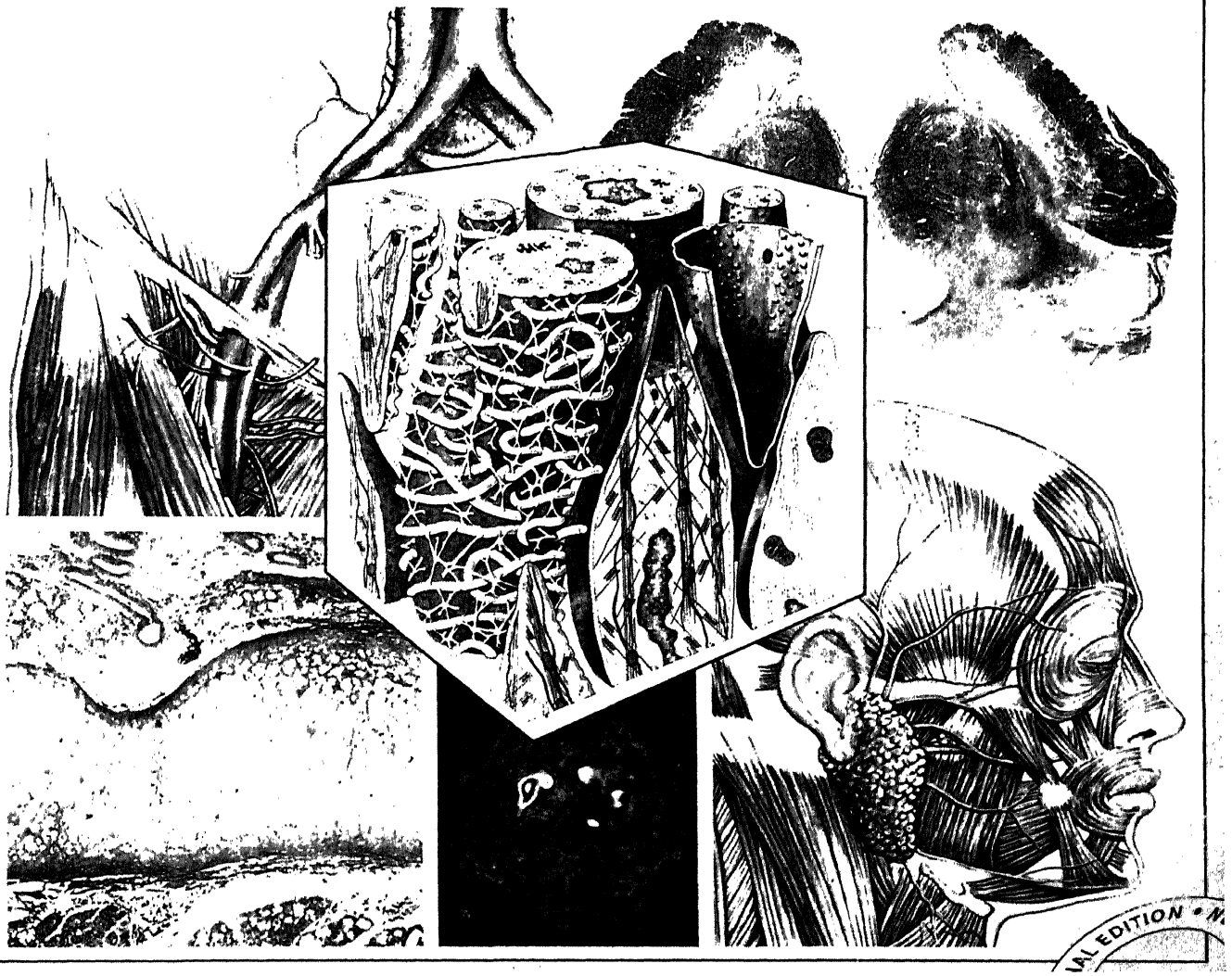
THIRTY-EIGHTH EDITION

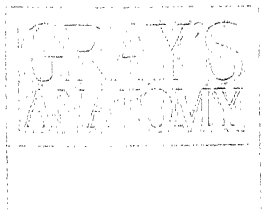
GRAY'S ANATOMY



CHURCHILL LIVINGSTONE

GRAY'S ANATOMY

CONTENTS



HAEMOLYMPHOID SYSTEM

Section Editor: Lawrence Bannister

With contributions from Dr Philip Shepherd, the complete revision of lymphocyte biology; Professor Marion Kendall, extensive revision and illustration of thymus structure and function; Dr Marta Perry, the new section on the palatine tonsil; and Dr Niall Kirkpatrick, the new section on the nasopharyngeal tonsil.

INTRODUCTION

The haemolymphoid system is a complex array of cells and tissues which are closely interdependent in their origins and functions in the body. The **haemal** component, arising in the bone marrow, provides: *red cells* (*erythrocytes*) which carry oxygen and to a lesser extent carbon dioxide between the lungs and general tissues; a wide variety of defensive cells, *leucocytes*, including the *neutrophil*, *eosinophil* and *basophil granulocytes*, and *monocytes* (*macrophages*), engaged in a plethora of defensive activities, and *platelets* which assist in haemostasis. Of these cells, only two, erythrocytes and platelets, are generally confined to the blood vascular system once they have entered it from haemopoietic tissue. All leucocytes possess the ability to leave the circulation and enter the extravascular tissues, the numbers of cells so doing being greatly increased in local infections and other disease conditions. The **lymphoid** component includes cells which are formed both in the bone marrow and in many sites outside it: the thymus, lymph nodes, spleen and lymphoid tissue associated with the alimentary tract and bronchi (lymphoid nodules). These produce different varieties of *lymphocytes*, which in the blood are included among the leucocytes and like the others of this class are able to migrate into extravascular sites although, unlike haemal cells, lymphocytes may also be found within the channels of the lymphatic system. Lymphocytes are concerned with various types of defence and indeed are the main source of the body's ability to resist infections. Included in lymphoid tissue are various types of phagocytic cell which, with the monocytes in the blood and some other phagocytic cells in other tissues (e.g. macrophages), are often considered as a distinctive component of the body, the *mononuclear phagocyte system* (p. 1414).

HAEMAL BLOOD CELLS AND TISSUES

BLOOD

Blood is an opaque turbid fluid with a viscosity somewhat greater than that of water (mean relative viscosity 4.75 at 18°C), and a specific gravity of about 1.06 at 15°C. When oxygenated, as in the systemic arteries, it is bright scarlet and when deoxygenated, as in systemic veins, it is dark red to purple.

Blood is a heterogeneous fluid consisting of a clear liquid, *plasma*, and formed elements, *corpuscles*; because of this admixture it behaves hydrodynamically in a complex fashion and belongs to that class of fluids termed non-Newtonian. This characteristic has important consequences in the physical study of blood flow in vessels (haemorrheology).

Plasma

Plasma is a clear, slightly yellow fluid which contains many substances in solution or suspension; the *crystalloids* give a mean freezing-point depression of about 0.54°C. Plasma is rich in sodium and chloride ions and also contains potassium, calcium, magnesium, phosphate, bicarbonate and many other ions, glucose, amino acids, etc. The *colloids* include the high molecular weight plasma proteins, composed chiefly of those associated with clotting, particularly prothrombin, the immunoglobulins and complement proteins involved in immunological defence (p. 1418), glycoproteins, polypeptides and steroids concerned with hormonal activities and globulins engaged in the carriage of hormones, iron and numerous other blood-borne substances. Since most of the metabolic activities of the body are reflected in the composition of the plasma, routine chemical analysis of this fluid has become of great diagnostic importance and a considerable body of information on its chemistry is available.

The formation of clots by the precipitation of the protein fibrin from the plasma is initiated by the release of specific materials from damaged cells and blood platelets (p. 1406) in the presence of calcium ions. If blood or plasma samples are allowed to stand, clot formation occurs to leave a clear yellowish fluid, the *serum*. Removal of the available calcium ions by means of citrate, various organic calcium chelators (EDTA, EGTA) and oxalate prevents clot formation in vitro; heparin is also widely used as an anticlotting agent, its action interfering with another part of the complex series of chemical interactions leading to fibrin clot formation.

Blood as a tissue

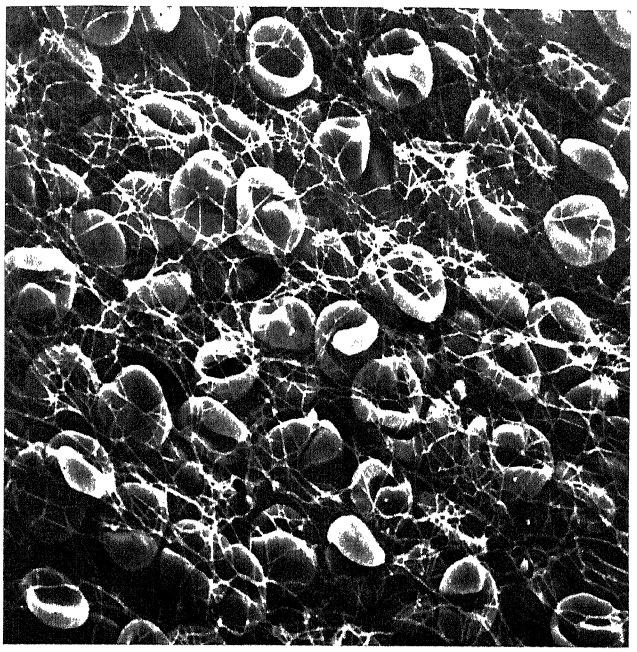
Blood has many affinities with connective tissue, as, for example, in the mesenchymal origin of its cells, the free exchange of leucocytes with the connective tissues and the relatively low cell : matrix ratio. The plasma substances and cells, however, arise from more than one source and so blood is really a composite tissue pool.

Formed elements of blood

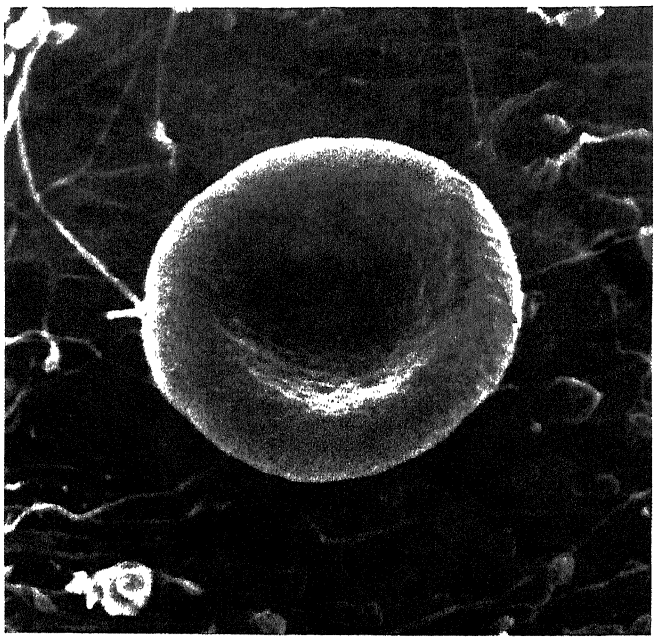
Blood contains three groups of formed elements: red and white blood cells and platelets. Some structural aspects of these elements are visible in fresh blood, but many others are seen only in fixed and stained specimens. The examination of blood cells is of considerable clinical importance since their numbers, proportions of different cell types and structure are valuable indicators of pathological changes in the body. Amongst other techniques, the Romanowsky methods of staining are particularly valuable and are widely used in clinical laboratories. These methods involve staining in aqueous solutions with methylene blue–eosin mixtures which colour both acidic dye binding and basic dye binding structures. The Giemsa and Leishman stains belong to this group. It should be noted that throughout this section, the figures given for cell dimensions and numbers are approximate ranges only. The data provided by different authorities vary somewhat; further, the dimensions of some cells when measured in the fresh state are substantially smaller than when measured in a dried smear; with erythrocytes the converse applies.

Erythrocytes (red blood cells, red blood corpuscles) form the greater proportion of the blood cells (99% of the total number), with a count of $4.1–6.0 \times 10^6/\mu l$ in adult males and $3.9–5.5 \times 10^6/\mu l$ in adult females. Each cell is a biconcave disc with a diameter in dried smear preparations of 6.3–7.9 μm (mean 7.1 μm) and a rim thickness of 1.9 μm; in wet preparations the mean diameter is 8.6 μm. Erythrocytes lack nuclei and are pale red by transmitted light, with paler centres because of their biconcavity. They show a tendency to adhere to one another by their rims to form loose piles of cells (*rouleaux*), a character determined by the properties of their cell coat. In normal

9.1 Fresh preparation of living erythrocytes showing rouleau formation and red pigmentation. Magnification × 500.



9.2 A scanning electron micrograph of erythrocytes, showing biconcave discoidal and other shapes; the filaments are fibrin resulting from clotting of the plasma after extravasation of blood. Magnification × 1500. (Photographed by Michael Crowder, Guy's Hospital, London.)



9.3 Scanning electron micrograph of an erythrocyte. Magnification × 7800.

blood a few assume a shrunken star-like, *crenated* form, a shape which can be reproduced by placing normal biconcave erythrocytes in a hypertonic solution, which results in osmotic shrinkage. Such cells are called *echinocytes* (Bessis 1973). In hypotonic solutions erythrocytes take up water and become spherical and may eventually lyse to release their haemoglobin (*haemolysis*); they are then termed *red cell ghosts* (erythrocytic umbrae).

Erythrocytes are bounded by a plasma membrane and consist internally mainly of a single protein, haemoglobin. The plasma membrane of erythrocytes has received much attention because of the ease with which it can be obtained for analysis in quantity (Bretscher & Raff 1975). It is about 60% lipid and glycolipid and 40% protein and glycoprotein. More than 15 classes of protein are present, including two major types. Firstly, the glycoproteins *glycophorins A, B* and *C* (each with a molecular mass of about 50 kDa) span the membrane, and their negatively charged carbohydrate chains project from the outer surface of the cell, conferring most of the fixed charge on the cell exterior by virtue of their sialic acid groups. Secondly, comes the 'Band 2' protein which may bear some antigenic groups; 'Band 3' protein is also a transmembrane macromolecule, constituting the important chloride channels in the erythrocyte membrane; these proteins are probably mainly present in the form of dimers, which are visible as intramembranous particles in freeze-fractured erythrocyte membranes; the ABO antigens (p. 1406) are all glycolipids (Race & Sanger 1975). Other proteins include several enzyme systems, some concerned with ionic regulation, others with the addition of lipid to the cell membrane from serum lipid. (This is necessary because the cell does not possess its own synthetic apparatus.)

The shape of the eythrocyte is largely determined by the protein *spectrin* (a name which reflects its biochemical preparation from red cell 'ghosts') which is attached to integral membrane proteins (Band 3) on the inner surface of the cell membrane via short lengths of actin filaments and other proteins (e.g. 'Band 4.2' protein, and *ankyrin*), forming a stabilizing network. This considerably stiffens the membrane, an effect which is aided by the large amount of cholesterol in the membrane itself. Red cells can thus regain their shape and dimensions after passing through the lumina of the finest ramules of the blood-vascular system; microscopic examination has shown that erythrocytes often pass through capillaries flattened face-first, buckling somewhat to a shield-like shape (Brånemark 1972) rather than rolling up, as might be expected.

Haemoglobin

Haemoglobin is a globular protein with a molecular mass of 67 kDa, consisting of *globulin* molecules bound to haem, an iron-containing *porphyrin* group. Each molecule is made up of four subunits, each in turn consisting of a coiled polypeptide chain with a cleft holding a single haem group. In normal blood, four types of polypeptide chain can occur, namely: α, β, γ and δ. Each haemoglobin molecule contains 2 α-chains and two others, so that several combinations and hence a number of different types of haemoglobin molecule are possible. Haemoglobin A (HbA), which is the major adult class, contains 2 α- and 2 β-chains. Haemoglobin A_2 (HbA2), a minor component in adults, is composed of 2 α- and 2 δ-chains. Haemoglobin F (HbF), found in fetal and early postnatal life, consists of 2 α- and 2 γ-chains. In the pathological condition *thalassaemia* only one type of chain is synthesized, so that a molecule may contain 4 α-chains (β-thalassaemia) or, more commonly, 4 β-chains (α-thalassaemia)—Haemoglobin H.

Each polypeptide chain is determined by a separate gene; a number of variant haemoglobins are known in which only one or a few amino acid residues are abnormal, reflecting slight alterations in the corresponding genes. In the Haemoglobin S of sickle-cell disease a single alteration in the β-chains (valine substituted for glutamine) causes a major alteration in the behaviour of the red cell and its oxygen-carrying capacity which, however, may confer some protection against malarial infection in areas where the disease is endemic. Other common variants include Haemoglobins C and D. The oxygen-binding power of haemoglobin is provided by the iron atoms of the haem groups, and these are always maintained in the ferrous (Fe^{++}) state by the presence of glutathione within the erythrocyte.

In addition to haemoglobin, erythrocytes possess a number of enzyme systems, notably those concerned with glycolysis and ionic transport, which together maintain low sodium levels within the cell against diffusion gradients, and thus create the appropriate conditions of pH and ionic strength for the normal functioning of haemoglobin. As intimated above, glutathione metabolism is also

active. Although, of course, in the absence of a nucleus and ribosomes, no protein synthesis takes place in mature erythrocytes, lipid in the plasma membrane can be replaced to some extent from circulating serum lipids, by the activity of membrane enzyme systems synthesized and placed in the red cell membrane during formation in the bone marrow.

The iron-containing compound *ferritin* is also often present in newly formed erythrocytes, as are also persisting remnants of the apparatus of protein synthesis (ribosomal and other RNAs) from the stage of differentiation of the cell in the bone marrow. After Romanovsky staining, the residual RNA of young erythrocytes causes a slight bluish tinge; with the supravital stain brilliant cresyl blue it forms a reticulum, giving the name *reticulocyte* to this type of cell. Later in maturation such evidences of basophilia disappear. Other inclusions may be present in red cells, particularly in pathological conditions; amongst these are nuclear remnants (*Howell-Jolly bodies*) and altered haemoglobin (*Heinz-Ehrlich bodies*).

Life span and destruction

Erythrocytes which have been labelled radioactively or antigenically and then injected into the circulation, have been shown to last between 100 and 120 days before being destroyed (Berlin et al 1959). As erythrocytes age they become increasingly fragile and their surface charges decrease as their content of negatively charged membrane glycoproteins is progressively reduced (Marikovsky & Danon 1969). The lipid content of the membranes also lessens. Aged erythrocytes are eventually ingested by the macrophages of the spleen and liver sinusoids, without previous lysis, and are then hydrolysed. Here, the haemoglobin is broken into its globulin and porphyrin moieties; the globulin is then further degraded into its constituent amino acids which pass to the general amino-acid pool of the body. The iron is removed from the porphyrin and can be used either directly in the synthesis of new haemoglobin in the bone marrow, or stored in the liver as ferritin or haemosiderin; the remaining haem portion is converted in the liver to bilirubin and is then excreted in the bile. The recognition of the senescent erythrocytes by macrophages appears to depend, at least in part, on the exposure of normal inaccessible parts of the Band 3 molecules in their membranes, causing auto-antibodies in the plasma to bind to them and sensitizing the cells to macrophage removal. Such altered, antigenic sequences are referred to as erythrocyte senescence antigens.

Red cells are produced by the bone marrow and are destroyed at the rate of about 5×10^{11} cells a day.

Fetal erythrocytes up to the fourth month of gestation differ markedly from those of adults, in that they are larger (10 μm), are nucleated and contain a somewhat different type of haemoglobin (HbF). From this time they are progressively replaced by the adult type of cell.

(9.4, 5)

Leucocytes (*white blood corpuscles* or *cells*) belong to at least five different categories, distinguishable by their size, nuclear shape and cytoplasmic inclusions (**9.5**). For practical convenience, these types of cell are often divided into two main groups, namely those with prominent stainable cytoplasmic granules, the granulocytes, and those without, the agranulocytes. However, in terms of their biology, this distinction is now known to be quite arbitrary.

Granulocytes. Also known as *granular leucocytes*, they all possess irregular or multilobed nuclei and are often termed *polymorphonuclear leucocytes* for this reason. This group is comprised of three types of cell, the granules of which give different staining reactions with the Romanovsky dyes: they are *eosinophil* leucocytes with granules which bind acidic dyes such as eosin, *basophil* leucocytes the granules of which bind basic dyes (methylene blue) strongly and *neutrophil* leucocytes whose granules stain weakly with both elements by a different type of reaction (see Wintrobe et al 1981; Zucker-Franklin et al 1981).

NEUTROPHIL LEUCOCYTES (9.4A, 5B)

Neutrophil polymorphonuclear leucocytes (*neutrophils*, *neutrophiles*, *heterophile leucocytes* or '*polymorphs*') form the largest proportion of the leucocytes (60–70% in adults, with a count of 3000–6000/μl). In dried smears, where the cells have flattened, they have a circular profile with a diameter of 10–15 μm. In the living state the cells may be spherical whilst passively circulating, but can flatten and become actively motile on contact with a suitable surface.

Within the cytoplasm the numerous granules give a variety of colour shades ranging from violet to pink when stained with Romanowsky stains such as Wright's and May-Grünwald-Giemsa, which are commonly employed in haematology. Under the electron microscope, too, the *granules* are heterogeneous in size, shape and content, but all are membrane-bound bodies containing hydrolytic and other enzymes. Two major categories can be distinguished according to their developmental origin and contents (Bainton et al 1971). Firstly, *non-specific* or *primary granules* which are formed early in neutrophil genesis (p. 1413); these are relatively large (0.5 μm) spheroidal lysosomes containing myeloperoxidase, acid phosphatase and several other enzymes. With light microscopy they stain strongly with neutral red and azure dyes and are thus said to be azurophilic. Secondly, *specific* or *secondary granules*, formed a little later, assume a wide range of shapes including spheres, ellipsoids and rods. These contain several substances with strong bactericidal actions, including alkaline phosphatase, aminopeptidase, lactoferrin and also collagenase, all of which are lacking in the primary granules. The secondary granules, however, lack peroxidase and acid phosphatase. Some enzymes such as lysozyme are present in both. The presence of these granules correlates well with the phagocytic activity of neutrophils.

In *mature neutrophil* granulocytes the nucleus is characteristically multilobate with up to six segments joined by narrow nuclear strands (the *segmented* stage). Less mature cells have fewer lobes, the earliest to be released under normal conditions being *juveniles* (*band* or *stab cells*) in which the nucleus is an unsegmented crescentic band. In certain clinical conditions, even earlier stages in neutrophil formation with indented or rounded nuclei (*metamyelocytes* or *myelocytes*, p. 1413) may be released from the bone marrow. In mature cells the edges of the nuclear lobes are often irregular; in females about 3% (range 1–17%) of the nuclei of neutrophils show a conspicuous 'drumstick' formation which represents the sex chromatin of the inactive X chromosome (*Barr body*; see also p. 63).

Mitochondria, a Golgi complex, a sparse endoplasmic reticulum and glycogen are present in the cytoplasm. A cytoskeleton of actin and myosin filaments is present, and a conspicuous array of microtubules is often seen between the 'arms' of the nucleus; it is interesting that locomotion of neutrophils is in the direction in which the free arms point. Neutrophil locomotion is dependent on actin myosin interactions (see p. 739).

Neutrophils form an important element in the defence systems of the body; they can endocytose microbes and particles in the circulation and, after migrating between the endothelial cells lining capillaries or venules, can perform local phagocytosis in the extravascular tissues, wherever it is needed. The engulfing of foreign objects is followed by digestion through fusion of the phagocytic vacuole, first with the *specific* granules, the pH being reduced to 4.0 by active transport of protons, then with the *non*-specific (azurophilic, primary) granules, which finish the process of bacterial killing and digestion. The chemical reactions occurring in these events have been intensively studied and prove to be quite complex. They involve the oxidation and addition of halide (chloride and iodide) radicals to the engulfed materials by means of enzymic action (myeloperoxidase, etc.) which have the effects of denaturing their proteins and other macromolecules. Lysozyme and lactoferrin are also highly toxic to bacteria. These processes require atmospheric oxygen for successful completion, so that neutrophils active in defence have a high oxygen demand. Phagocytosis, or the release of granules, may be enhanced by the presence of antibodies attached to the surfaces of neutrophils which can bind specifically to target antigens, for example in a type of bacterium to which the body has previously been exposed. Opsonizing antibodies (*opsonins*, p. 1420) coating the antigenic target may also promote phagocytosis. The antibodies in both cases are secreted by lymphocytes (p. 1420), and the neutrophil granulocyte is just one in a series of defensive cells which form an interrelated system for the elimination of foreign materials from the tissues.

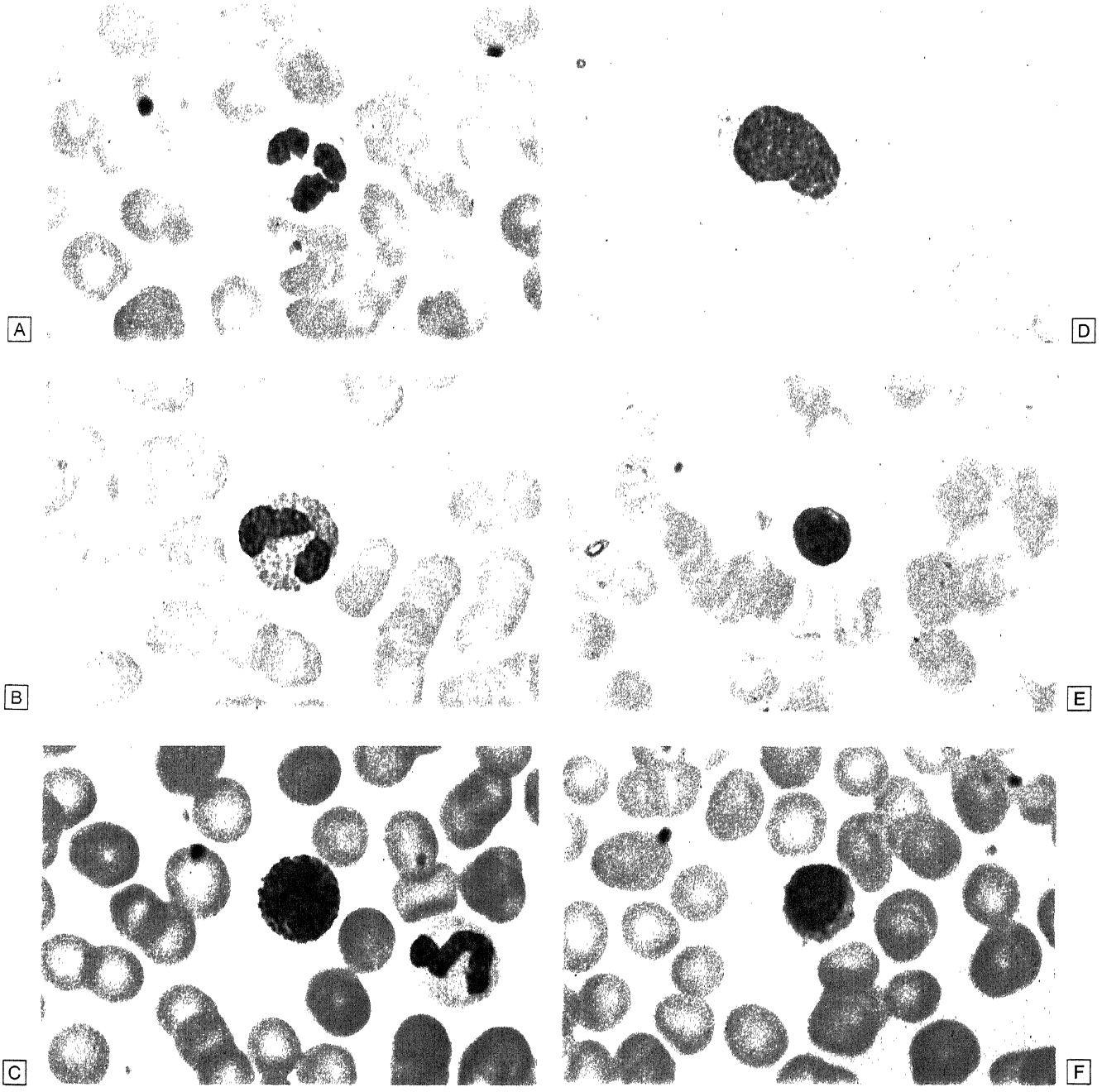
After phagocytosis, the neutrophil's cytoplasmic granules gradually become used up, so that a marked reduction in their number (degranulation) occurs. Granules may also be discharged from the

surface of the cell when it is suitably stimulated, to damage or kill neighbouring organisms or cells. Inappropriate release of such enzymes is associated with various pathological conditions, for example rheumatoid arthritis, where tissue destruction and chronic inflammation may result.

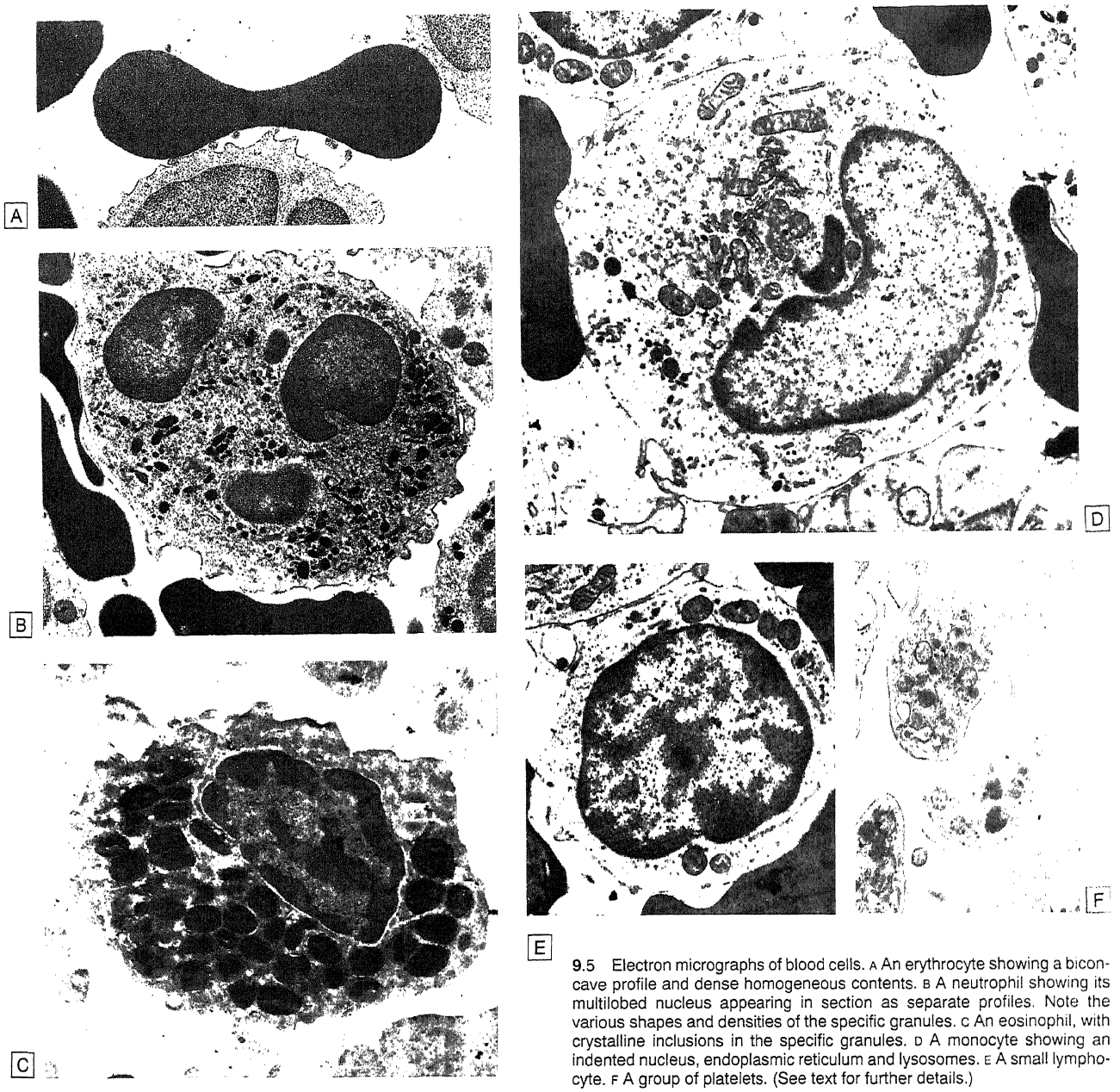
The numbers of circulating neutrophils vary considerably, often rising during episodes of acute bacterial infection. They may circulate freely in the blood (the *circulatory pool*), or they may adhere to the walls of postcapillary venules and other vessels (the *marginal pool*) to re-enter the circulation when suitably recruited, for example during brief exercise or by exposure to noradrenaline. However, neutrophils are short-lived (half-life 7.5 hours); they may either be destroyed in the bloodstream, or pass through the endothelial walls to the extravascular tissues, engaging in defence; alternatively, after entering various secretory ducts such as the bronchi, salivary gland ducts and the urinary tract, they are lost to the body. Their surfaces bear a number of well-characterized markers, including a number of adhesion molecules important in the migration of neutrophils through endothelia.

EOSINOPHIL LEUCOCYTES (9.4B, 5B)

Eosinophil leucocytes are similar in size, shape and motile capacity to neutrophils but are present only in small numbers in normal blood (100–400/μl). Their cytoplasmic granules are uniformly large (0.5 μm) and give the living cell a slightly yellowish colour; with



9.4 Blood cell types stained in smeared preparations by the Giemsa method. Erythrocytes are shown in all figures, which also demonstrate other cell types. A Neutrophil leucocyte and platelets. B Eosinophil leucocyte. C Basophil leucocyte with prominent densely staining cytoplasmic granules and neutrophil leucocyte. D Monocyte. E Small lymphocyte. F Medium-sized lymphocyte. (Material provided by J P Black, Department of Haematology, Guy's Hospital.)



9.5 Electron micrographs of blood cells. A An erythrocyte showing a biconcave profile and dense homogeneous contents. B A neutrophil showing its multilobed nucleus appearing in section as separate profiles. Note the various shapes and densities of the specific granules. C An eosinophil, with crystalline inclusions in the specific granules. D A monocyte showing an indented nucleus, endoplasmic reticulum and lysosomes. E A small lymphocyte. F A group of platelets. (See text for further details.)

Romanowsky stains they are uniformly orange to red (**9.4**). The nucleus has two prominent lobes connected by a thin strand. Ultrastructurally (Zucker-Franklin 1980, 1985), the cytoplasm is seen to be packed with specific granules (**9.5**) which are spherical or slightly ellipsoidal. Each of these bodies is bounded by a membrane and contains an amorphous material (the *matrix* or *externum*) in which is embedded a prominent crystal (the *crystalline core* or *internum*); in human eosinophils the crystalline core shows a square lattice structure with a 4 nm repeat pattern. The matrix contains several lysosomal enzymes including acid phosphatase, ribonuclease, phospholipase and a myeloperoxidase unique to eosinophils. The crystalline core is composed of the characteristic *major basic protein*, of about 9.2 kDa in molecular weight. This protein contains a high ratio of arginine residues which give the granules their strong eosinophilic staining properties. Apart from these granules, the cytoplasmic organelles are similar to those of neutrophil granulocytes.

Like other leucocytes, eosinophils are able to pass into the extravascular tissues from the circulation when suitably stimulated. In small numbers they are typical constituents of the dermis and of connective tissue components of the bronchial tree, alimentary tract, uterus and vagina and thymic medulla. The total life span of these cells is a few days, of which about 30 hours is spent in the circulation and the remainder in the extravascular tissues.

The functions of eosinophils are not yet fully understood. Their ratio to other leucocytes rises greatly (an *eosinophilia*) in certain allergic disorders, and also in worm infestations, and there is now much evidence that they play an important part in the immune system, phagocytosing and inactivating antigen antibody complexes and also various inflammatory substances, for example histamine and leukotrienes I and II so they may be important in limiting the effects of these substances on the surrounding tissues. It has also been shown in vitro that they can attach themselves to and kill parasitic schistosome larvae which invade the circulation.

BASOPHIL LEUCOCYTES (9.4c, 5c)

Like other granulocytes, these are 10–15 μm in diameter (**9.4**), but

form only 0.5–2% of the total leucocyte population of normal blood, with a count of 25–200/μl. Their distinguishing feature is the presence of large, conspicuous basophilic granules (**9.4c**), varying in number from 10 to 100 per cell. The nucleus is somewhat irregular but not usually lobated, unlike those of the other granulocytes. Ultrastructurally, the basophilic granules are membrane-bound vesicles with densely stained contents showing a variety of crystalline, lamellar and granular inclusions. Heparin, histamine and several other inflammatory agents are contained in these granules, which closely resemble those of mast cells (p. 79). These substances are apparently associated with polysaccharides, since the granules are also positive to carbohydrate stains, for example periodic acid-Schiff (PAS) and Azure A, with which they are *metachromatic*, i.e. they stain a different colour from that of the dye. Their life span in the circulation is long (9–18 months in mice).

Although they resemble mast cells (Selye 1965) and, like these, are formed in the bone marrow, there is much evidence that many basophils represent a different, albeit closely related, cell line peculiar to the circulation, as shown by their reactions with monoclonal antibodies and differences in cell development (see also p. 1414). It is likely, however, that they include true mast cell precursors en route from the bone marrow to the extravascular tissues. Their functions in the circulatory system are at present rather obscure.

MONOCYTES (9.4D, 5D)

Monocytes are the largest of the agranular leucocytes (15–20 μm in diameter in smears) but they form only a small proportion of the total leucocytes (2–8% with a count of 100–700/μl of blood). The nucleus, which is euchromatic, is relatively large and has a characteristic indentation on one side. The cytoplasm forms a wide rim around the nucleus, near the indentation of which lies a prominent Golgi complex and vesicles stainable with neutral red. Ultrastructurally (Cawley & Hayhoe 1973; Zucker-Franklin et al 1981), many lysosomes are seen to be present, together with some peripheral rough endoplasmic reticulum. Mitochondria are quite abundant and the skeleton is well represented, reflecting the highly motile nature of the cell. Monocytes are actively phagocytic.

Their surfaces express Class II major histocompatibility complex (MHC) antigens and various CD44 markers which show that they belong to the mononuclear phagocyte family, and are indeed identical to macrophages. It would appear that monocytes are macrophages in the process of passing from the bone marrow, where they are formed, to the peripheral tissues via the bloodstream, these cells passing into extravascular sites through the walls of capillaries and venules. This topic is discussed further on pages 1414–1415.

LYMPHOCYTES (9.4E, F, 5E, 13–15)

Lymphocytes (**9.4, 5**) are the second most numerous type of leucocyte, forming 20–30% of their total number, i.e. 1500–2700/μl of blood. Like other leucocytes, they are also found in extravascular tissue, but they are remarkable in being formed in large numbers outside the bone marrow as well as within it. They therefore constitute a widely distributed *lymphoid* system (see p. 1417 for a fuller description).

Structural classification

Morphologically, in the blood, the term 'lymphocyte' refers to agranular leucocytes 5–15 μm in diameter. This size range represents a heterogeneous collection mainly of B and T lymphocytes in different stages of activity and maturity. About 85% of all circulating lymphocytes in normal blood are T cells. Included under the 'lymphocyte' heading are also a small percentage of cells ('null cells') which are not apparently true lymphocytes at all but either related cells (e.g. natural killer cells) or some other quite different form, such as circulating haemopoietic stem cells, antigen-presenting cells, and precursors of osteoclasts and chondroclasts in the process of passing from the bone marrow to other tissues. The various types and conditions of cells can now be at least partially separated by means of immunocytochemical techniques, involving the use of monoclonal antibodies (see p. 1420) raised against different components of their cytoplasm. Where these detect specific macromolecules exposed at the surface of lymphocytes, living cells can also be separated into different classes by various techniques such as the fluorescence cell sorter.

In this account the B and T lymphocytes will form the main focus of description, except where otherwise stated. The terms B lymphocyte or T lymphocyte will all be used interchangeably with B cell and T cell respectively.

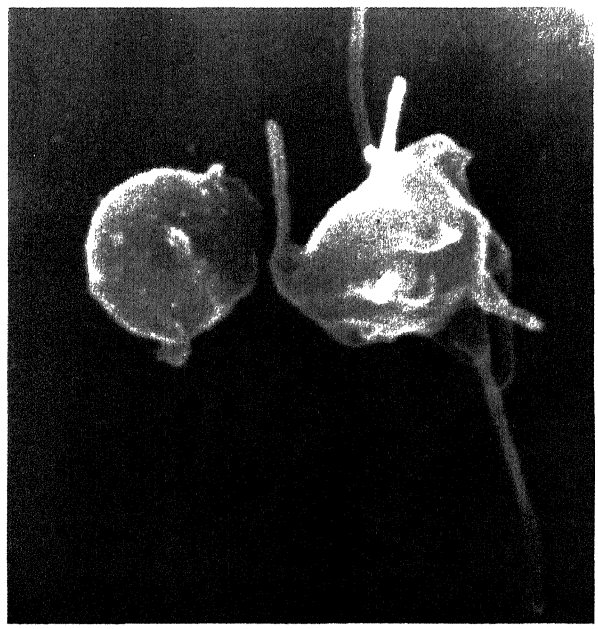
Small lymphocytes (both B and T cells) form the majority of lymphocytes in circulating blood. These are by definition within the size range 5–8 μm (some authorities extend this to 10 μm). Each cell contains a rounded densely staining nucleus, surrounded by a very narrow rim of cytoplasm. The nucleus contains dense, coarse bands of chromatin (a leptochromatic nucleus: *leptos* = a thread), and one or more small nucleoli. The cytoplasm stains slightly blue with Romanovsky dyes. Under the electron microscope (**9.5E, 15**), few organelles can be seen in the cytoplasm, apart from a small number of mitochondria, single ribosomes (monosomes), sparse profiles of endoplasmic reticulum and occasional lysosomes; these features indicate a low metabolic rate and such lymphocytes are said to be in the 'resting' phase. However, these cells are freely motile when they settle on to solid surfaces, and can pass between endothelial cells to exit from or enter the vascular system. They may make extensive migrations within the various tissues, including the epithelia, even passing into the body's secretions such as saliva.

Larger lymphocytes 9–15 μm in diameter, again belonging to both B and T cell classes, constitute within the circulation a mixture of very immature cells (lymphoblasts) capable of cell division to produce small lymphocytes, and maturing or mature cells which have become functionally active after stimulation by the immune system, for example in the presence of antigens (see p. 1407). Both lymphoblasts and maturing cells are actively engaged in synthesizing proteins, and thus contain a nucleus which is at least in part euchromatic, and a basophilic cytoplasm with numerous polyribosome clusters. The ultrastructure of these cells varies according to their class and will be described where appropriate (see below).

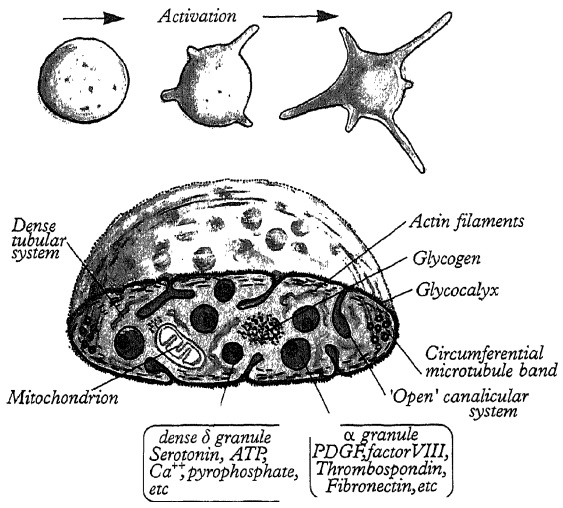
The life span of lymphocytes ranges from a few days to perhaps many years and so we can distinguish between *short-lived* and *long-lived* lymphocytes, the latter being of some significance in the mechanisms of *immunological memory* (but see p. 1421).

PLATELETS (9.6, 7)

Blood platelets, also known as *thrombocytes* (**9.5, 6**), are relatively small (2–4 μm across) irregular or oval discs present in large numbers (250 000–500 000/μl) in blood. In freshly taken blood samples they



9.6 Scanning electron micrograph of platelets with extending filopodia. Magnification × 10 00.



9.7 Diagram of the internal organization of a platelet and shape changes on activation.

readily adhere to each other and to all available surfaces, unless the blood is treated with citrate or other substances which reduce the availability of calcium ions. In Romanowsky-stained preparations, platelets show an outer clear zone (*hyalomere*) and in inner basophilic (or azurophilic) granular region (*granulomere*). Ultrastructurally (Cawley & Hayhoe 1973; Zucker-Franklin et al 1981), each platelet is seen to be anucleate, unlike similar cells in submammalian vertebrates (for which the term *thrombocytes* is perhaps best reserved). Each platelet is surrounded by a plasma membrane bearing a thick glycoprotein-rich coat, responsible for the adhesive properties of platelets. Beneath the surface is a band of about 10 microtubules which runs around the perimeter of the cell and probably determines its shape. Associated with these are actin filaments, myosin and other proteins related to cell contraction. Within the cytoplasm are also mitochondria, glycogen, a few profiles of agranular endoplasmic reticulum, including some narrow tubular channels and tubular invaginations of the plasma membrane and various membrane-bounded vesicles. These vesicles include three major types, designated alpha, delta and lambda granules.

Alpha-granules are the most prominent, with diameters of up to 500 nm; they contain platelet-derived growth factor (PDGF), fibrinogen and other substances. In the smaller (300 nm) *delta*-granules are 5-hydroxytryptamine (serotonin), concentrated from the blood plasma by endocytosis; calcium ions, adenosine 5′-diphosphate (ADP), adenosine 5′-triphosphate (ATP) and pyrophosphate are also present. In the smaller (250 nm) *lambda* set, granules contain several lysosomal enzymes (Bentfield & Bainton 1975).

Platelets are important in haemostasis (the limiting of bleeding after injury to vessels) and a number of more general, systemic functions, which at present are rather poorly understood. When a blood vessel is damaged, platelets first aggregate at the site of injury, assisting to staunch the wound with a *platelet plug*. Adhesion between platelets (agglutination) and to other tissues is a function of the thick platelet coat and is promoted strongly by the release of ADP and calcium ions from the platelets as a response to vessel injury. Then, substances released from the alpha-granules, together with factors released from the damaged tissues, initiate a complex sequence of chemical reactions in the blood plasma, leading to the precipitation of insoluble *fibrin* filaments, generating a three-dimensional meshwork, the *fibrin clot*, to which more platelets attach, inserting extensions of their surfaces (filopodia) deep into the spaces between the filaments, to which they adhere strongly. Next, the platelets contract (*clot retraction*) by actin–myosin interactions within their cytoplasm (p. 45), concentrating the fibrin clot and pulling the adhering walls of the blood vessel together, limiting further any leakage of blood. Eventually, on repair of the vessel wall, *clot removal* occurs as a result of complicated enzymic activities, including those of *plasmin* formed by plasminogen activators in the plasma, and probably assisted by lysosomal enzymes derived from the small (lambda) granules of platelets.

Platelets may also be involved in various other biological activities; for example they have receptors for class IgE antibody molecules which enable them to adhere to and damage antibody-coated targets by means of their lysosomal secretions. PDGF is also a potent trophic substance in laboratory cell cultures and may also exert a similar effect elsewhere, for example in regenerating tissues after damage (see Longenecker 1985).

Over 300 red cell antigens are recognizable with specific antisera, and the other cell types also carry many antigens of similar abundance. Antigens which are expressed by alleles at a simple locus or at loci which are closely linked are termed a *blood group system*.

Early attempts at transfusion of blood led to the discovery that erythrocytes bear antigens on their surface (see Race & Sanger 1975) which can interact with naturally occurring antibodies in the plasma of other individuals, causing agglutination and lysis of the erythrocytes. Such antigens, which are not shared with all members of a particular species, are termed *iso-antigens*; other iso-antigens are found amongst cells of other tissues (see below). Erythrocyte antigens are known as *agglutinogens* and the corresponding antibodies are *agglutinins*.

Erythrocytes from an individual can bear several different types of antigen, each type belonging to an antigenic system in which a number of alternative antigens are possible in different persons. So far 19 major groups have been identified, which vary in their frequency of distribution amongst the various races of mankind, including the ABO, Rhesus, MNS, Lutheran, Kell, Lewis, Duffy, Kidd, Diego, Cartwright, Colton, Sid, Scianna, Yt, Auberger, Ii, Xg, Indian and Dombrock systems. Clinically, only the ABO and Rhesus groups are of major importance. Red cells also bear various other minor antigens, some of them very infrequent. Some blood group antigens are also expressed in other tissues or secretions; for example olfactory receptors and salivary glands express the ABO antigens.

All of these antigens are determined by genes carried by autosomes, except Xg which is borne by the X chromosome. Within each group the antigens are determined by alleles and inheritance is in accordance with simple mendelian principles. Thus, in the ABO system the genome may be homozygous and carry the AA complement, the blood group being A, the BB complement giving blood group B; or it may carry neither (OO), the blood group consequently being O. In the heterozygous condition the following combinations can occur: A B (blood group AB), A O (blood group A) and B O (blood group B). In Caucasians and Negroes group O is the commonest, being present in about 50% of the population, followed in frequency by groups A, B and AB in that order (Mourant 1975). The ABO alleles are carried on chromosome 9. In West Africans the Duffy determinant (Fy) is almost always absent, a lack which confers resistance to *Plasmodium vivax* malaria (Miller & Carter 1976).

The plasma in each case carries naturally occurring antibodies specific to the antigens which are not present on the erythrocytes in the same blood, so that in group A blood, anti-B antibodies are found. Similarly, present in group B blood are anti-A antibodies, in Group O blood both anti-A and anti-B antibodies and in group AB blood there is neither type of antibody.

Transfusions succeed only if the recipient's antibodies do not correspond to the donor's antigens and cross-matching of blood antigens is therefore vitally important. Persons with group AB blood, lacking antibodies to both A and B antigens, can be transfused with blood of any group and are termed *universal recipients*; conversely, those with group O, *universal donors*, can give blood to any recipient, the donor's antibodies being diluted to insignificance. Normally, however, blood is only transfused between persons with precisely corresponding groups, since anomalous antibodies of the ABO

system are occasionally found in blood and may cause agglutination or lysis.

Within the ABO system several subgroups exist (A_1, A_2, A_{1B}, etc.); the cross-matching of some of these is important in transfusions. The anti-ABO agglutinins, like all others (except those of the Rhesus system), belong to the immunoglobulin M (IgM) class and do not cross the placenta during pregnancy.

Rhesus antigen system

The Rhesus antigen system, so-called because of its presence also in the erythrocytes of the Rhesus monkey, is determined by three sets of alleles, namely Cc, Dd and Ee, the most important clinically being Dd; all are carried on chromosome 1. The commonest condition in the UK is CDe and about 83% of the population is Rhesus-positive. Inheritance of the Rh factor obeys simple mendelian laws and it is therefore possible for a Rhesus-negative mother to bear a Rhesus-positive child. Fetal Rh antigens can, under these circumstances, stimulate the production of anti-Rh antibodies by the mother and since these belong to the immunoglobulin G group of antibodies they are able to cross the placental barrier and cause agglutination of fetal erythrocytes. In the first of such pregnancies little damage is usually caused, but in subsequent Rh-positive ones massive destruction of fetal red cells may ensue, causing fetal or neonatal death. Sensitization of the mother's immune system can also result from abortion or miscarriage, or even occasionally amniocentesis, which may introduce fetal antigens into the mother's circulation (see Contreras & Hewitt 1989). Treatment is by exchange transfusion of the neonate infant or, prophylactically, by desensitizing the mother after the first Rh-positive pregnancy with Rh-immune serum, which appears to destroy the fetal Rh antigen in the maternal circulation before the processes of immunological memory can be entrained (see Clarke 1975).

Of the other antigenic systems known, some of which are occasionally of clinical importance, many are restricted to individual genic groups or even families; they can be of great value to anthropologists when tracing demographic relationships, as of course are the major systems described above.

Other antigenic systems such as MNS (shared with other tissues in the body) can be used in medicolegal investigations to establish identity of blood, or in parental identification. These antigens can remain intact long after death, and have been detected even in mummified tissues from Egypt over 4000 years old (Harrison et al 1969). The Lewis system is not synthesized by the red cell lineage itself, but is absorbed from the plasma; it is a secretory product of salivary and other glands.

The genetics of blood groups is complicated by gene linkage with other characters which may be of some clinical importance; for example duodenal ulcers show a higher incidence in those with group O blood than in the general population.

Leucocytes also bear antigens and about 12 such groups have so far been identified, 10 of them belonging to the same complex system. These are similar to the *histocompatibility antigens* involved in graft rejection. For further details of the blood groups, see Race and Sanger (1975); Mollison et al (1987); Contreras and Lubenko (1989).

HISTOCOMPATIBILITY ANTIGENS

Amongst important components exposed at the surfaces of most cells are the molecules which determine the individuality of tissues from different persons (the histocompatibility locus antigens) and which are intimately related to the functioning of the immune system. They also share the same region on one of the chromosomes, their genes being grouped together as the Major Histocompatibility Complex (MHC), expressed in the body as a number of distinctive proteins. This system has come into prominence because of its importance in tissue grafting and transplant surgery, and various other clinical approaches.

In humans, the MHC genes lie on the short arm of chromosome 6 and are grouped in a linear sequence close to each other; crossing over during meiosis rarely or never occurs in this sequence, due to either their proximity to each other or some other intrinsic resistance to this process. The MHC region expresses a number of distinctive classes of molecules in various cells of the body, the genes being, in order of sequence along the chromosome: the Class I, II and III MHC genes. Class I consists of the histocompatibility locus antigen (HLA) genes, subdivided into A, B and C subregions, A and B being represented by a number of different alleles; these genes are capable of generating throughout the cells of the body a distinctive set of gene products, expressed at the surfaces of most cells. These appear to be different in all individuals, conferring a unique chemical identity which is the basis of the immune reactions in the body, since lymphocytes recognize and attack cells bearing alien antigens mainly when these are present alongside characteristic 'self'-HLA molecules. This enormous range of possible alternatives is a result of the substructure of the genes coding for them, which are subdivided into many smaller units; these can be spliced together in many different combinations, in a manner resembling the mechanisms for creating diversity amongst antibodies or T-cell receptors (pp. 1421, 1422), although of course there is a fundamental difference in the outcome, since only one HLA sequence out of a great possible range is selected during development. The HLA sequence also has interesting diagnostic aspects because of chromosomal linkage to sites affecting the frequencies of certain diseases (see below).

The genes for Class II MHC molecules include three sub-divisions termed DR, DQ and DP. Of their products, the MHC-DR molecules are best known; these occur on the surfaces of antigen presenting cells generally classed as macrophages (p. 1420) and including various dendritic cells of lymphoid tissue (e.g. follicular dendritic cells, interdigitating cells) and the epidermis (Langerhans cells). These molecules are anchored into the membrane and like the HLA antigens are highly polymorphic (i.e. have different chemical structures in different individuals). They can bind to alien antigens which the cells have phagocytosed and partially digested, and this combination is presented to helper or cytotoxic T cells where they temporarily bind to the CD 3 molecules (T-cell receptors) present on the lymphocyte surface to activate that cell (p. 1422). The genes for Class III MHC products are expressed in various components of the complement system, as well as some other, non-immune related proteins.

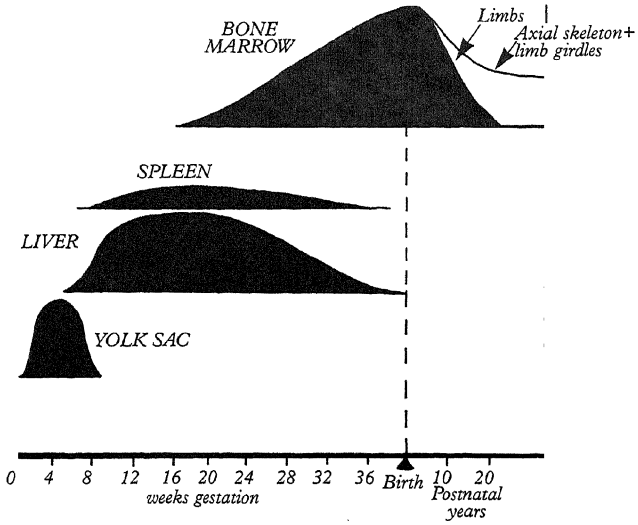
The HLA system has attracted much attention because of its importance in transplant surgery and, in a quite different way, because of the association between certain of its subgroups and some types of disease. For example, the subtype HLA-B27 is present in nearly all cases of ankylosing spondylitis; the condition of haemochromatosis, a disturbance of iron metabolism, is strongly associated with $HLA\text{-}A_3$ and rheumatoid arthritis with $HL_4\text{-}D_4$. Other conditions that display a statistical correlation with the HLA system include diabetes in juveniles, Hodgkin's disease and multiple sclerosis. The reasons for these associations are not clear, but are thought to represent some type of loose *genetic linkage* between the HLA determinants and other alleles predisposing the body to a range of diseases. In mice, where this system has been widely investigated, genes that apparently control the responsiveness of the immune system to infection (the Immune related, or Ir genes) are situated between those genes that determine the major histocompatibility (H2) factors.

HAEMOPOIESIS

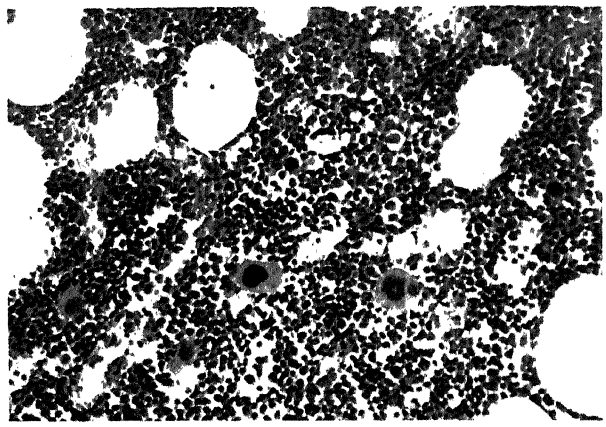
EARLY DEVELOPMENT OF HAEMOPOIETIC TISSUE

The earliest sign of blood *vessel* formation in the human embryo is when, during the early primitive streak stage of development, angioblastic tissue differentiates almost simultaneously in various extraembryonic sites, namely, in the mesenchyme of the yolk sac wall and in similar tissue of the connecting stalk and chorion. The earliest formation of blood *cells* (**9.6**), however, is confined to the wall of the secondary yolk sac, where they differentiate from deeply placed mesenchymal cells which lie next to the yolk sac endoderm. Whilst a mesodermal or endodermal origin for these cells is still debated, they are unquestionably mesenchymal in character and differentiate into the primitive *stem cells* of the haemopoietic line, which give rise directly to fetal blood cells.

Beginning in the second month, a number of intraembryonic sites of haemopoiesis appear and slowly replace the earlier sites. These intraembryonic sites succeed but overlap each other in time, each site gradually increasing in importance and then waning. Initially,



9.8 Diagram showing the sites of early haemopoiesis.



9.9 Photomicrograph of a section of bone marrow from a fetal human long bone. Note the heterogeneous collection of cell types including four large megakaryocytes. Magnification × 150.

the intraembryonic sites are broadly *intravascular*, but soon *extravascular* loci of haemopoiesis supervene. Rapidly the *liver* becomes the dominant organ of embryonic blood formation (the *hepatic phase*), its activities continuing until about the seventh month. Lagging somewhat behind the liver, the *spleen* is then added, its haemopoiesis continuing from the third to sixth months. From late in the month, an additional source of blood cells emerges, namely the *bone marrow* (*myeloid tissue*) where **all** blood cell types are formed, and later the peripheral lymphoid tissues, where only lymphocytes are produced (lymphopoiesis). The *thymus* is an active lymphopoietic

9.10 Microscopic organization of bone marrow depicting a sinusoid in section, showing haemopoietic islands centred on macrophages (yellow), forming erythrocytes (red), various classes of leucocytes (blue), megakaryocytes (beige), adipocytes (orange), fibroblasts (brown) and endothelial cells (dark blue-purple) flanked by a basal lamina and reticulin fibres (white). A group of platelets (white) and various other cellular types are shown passing through apertures in the endothelial linings of sinusoids. An arteriole is also depicted (top left).

organ from this stage (whilst initially it also has some general haemopoietic functions). The myeloid and lymphoid tissues become the dominant source of supply by the seventh month and shortly after birth all other sites have regressed completely. Occasionally, clumps of tissue capable of total haemopoiesis are found outside the bone marrow (*extramedullary tissue*) in paravertebral sites; in pathological cases, where there is more demand for haemopoiesis, and especially during early childhood, complete haemopoiesis may also occur in the liver, spleen and lymph nodes and infrequently also in the kidneys, adrenals, adipose tissue, general connective tissue and even in cartilage. In other species, such as mouse, haemopoietic sites may normally persist in small foci within extramyeloid tissues, for example the spleen and thymus.

BONE MARROW (9.9, 10, 13)

Bone marrow is a soft pulpy tissue which is found in the marrow cavities of all bones and even in the larger Haversian canal of lamellar bone. It differs in composition in different bones and at different ages and occurs in two forms, *yellow* and *red marrow*.

During fetal life and at birth there is red marrow throughout the skeleton. After about the fifth year the red marrow is gradually replaced in the long bones by yellow marrow. The replacement commences earlier and is more advanced in the more distal bones. Further, in each bone the replacement, in general, proceeds from the distal to the proximal end, though some maintain that it commences in the centre of the shaft and extends in both directions, but more rapidly in the distal. By 20–25 years of age the red marrow persists only in the vertebrae, sternum, ribs, clavicles, scapulae, pelvis, cranial bones and in the proximal ends of the femora and humeri. In old age the marrow of the cranial bones undergoes degeneration and is then termed *gelatinous marrow*.

Yellow marrow

The yellow marrow consists of a basis of connective tissue, supporting numerous blood vessels and cells, most of which are fat cells, although a small population of typical red marrow cells persists and may be reactivated when the demand for blood cells becomes sufficiently great, as noted above.

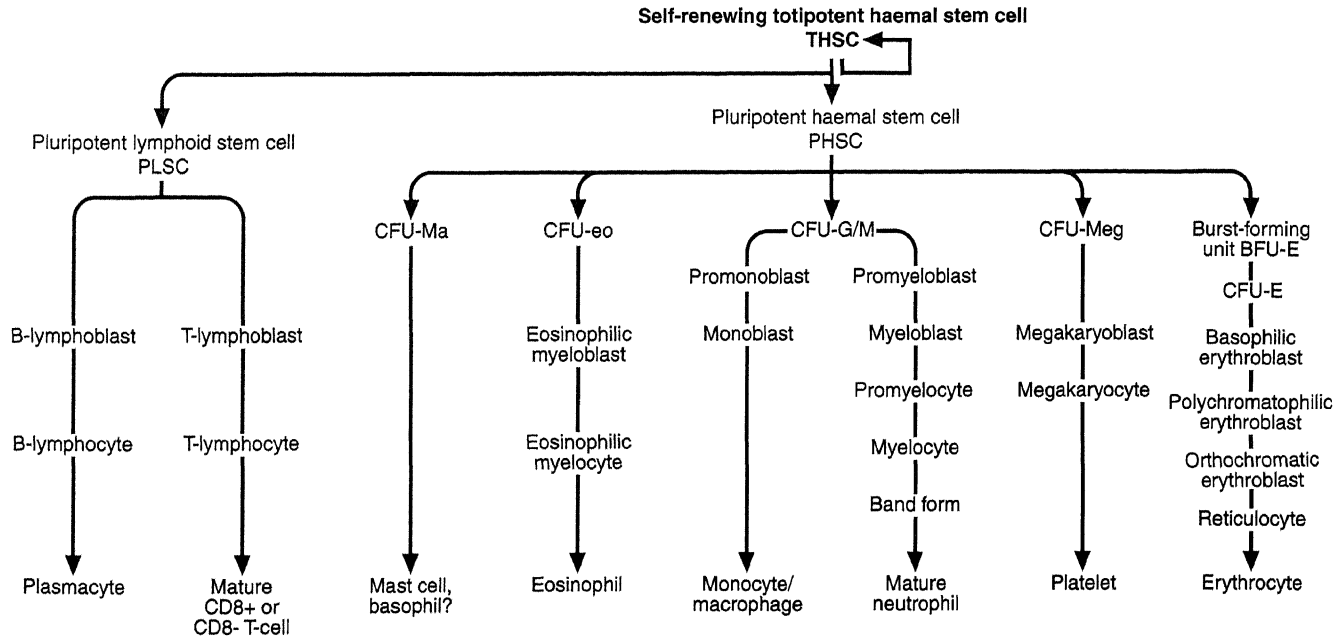
Red marrow (9.9, 10)

The red bone marrow consists of a network of loosely woven connective tissue, the *stroma*, supporting clusters of haemopoietic cells (*haemopoietic cords* or *islands*) and a rich vascular supply in which large, thin-walled *sinusoids* are prominent (for reviews see Quesenberry & Levitt 1979; Tavasolli & Yoffey 1983; Weiss 1984; Golde & Takaku 1985). The stroma also contains a variable amount of fat, depending on age, site and the haematological conditions of the body; small patches of lymphoid tissue are additionally present. Thus, the marrow consists of two major compartments, one vascular and the other extravascular. The whole assembly is enclosed within a bony framework, from which it is separated by a thin layer of bone-lining cells (p. 459).

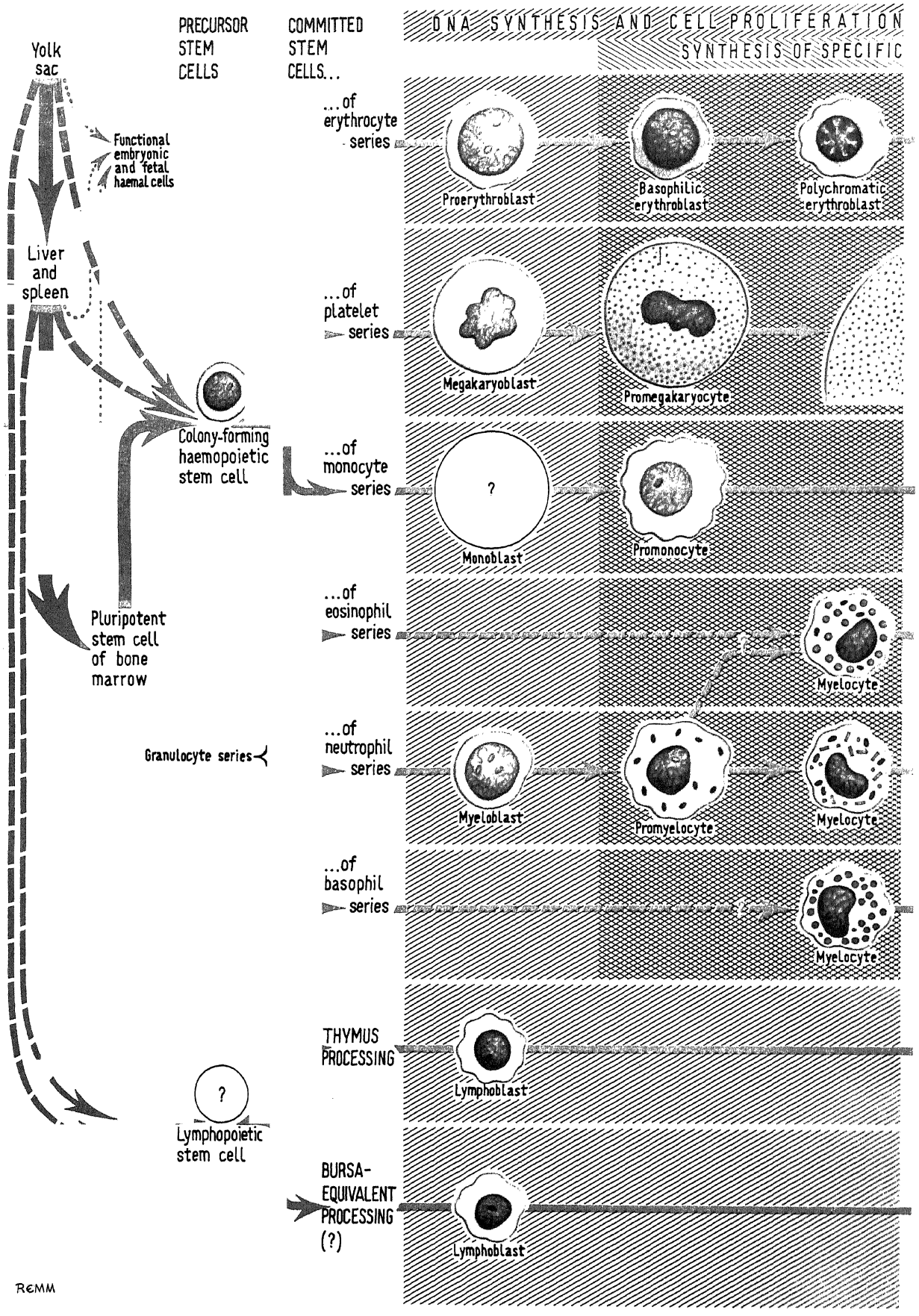
Stroma. This is composed of a delicate network of fine collagen (reticulin) fibres secreted by and adherent to highly branched *adventitial reticular cells* which appear to be a type of fibroblast and derived from embryonic mesenchyme. When haemopoiesis ceases, as in some limb bones in adult life, these adventitial cells become distended with fat globules, filling the marrow with yellow fatty tissue; but if there is a later demand for haemopoiesis, these cells can change back to their earlier stellate form. Also in the stroma are many *macrophages* attached to stromal fibres, some of them being embedded in the centres of haemopoietic cell clusters. These cells are actively phagocytic of cellular debris created by haemopoietic development, especially the extruded nuclei of erythroblasts and remnants of megakaryocytes, but they also have a major role in the control of haemopoietic cell differentiation, proliferation and maturation (see below).

Endothelial cells line the marrow sinusoids, the single layer of cells being supported by reticulin fibres on its basal surface. Endothelial cells are interconnected by tight junctions, which appear to be effective barriers between vascular and extravascular spaces. The passage of newly formed haemal cells from the haematopoietic compartment into the bloodstream occurs through temporary apertures (large fenestrae) formed in the endothelial cell cytoplasm, the migrating cell fitting tightly as it passes through, and the aperture closing immediately behind it.

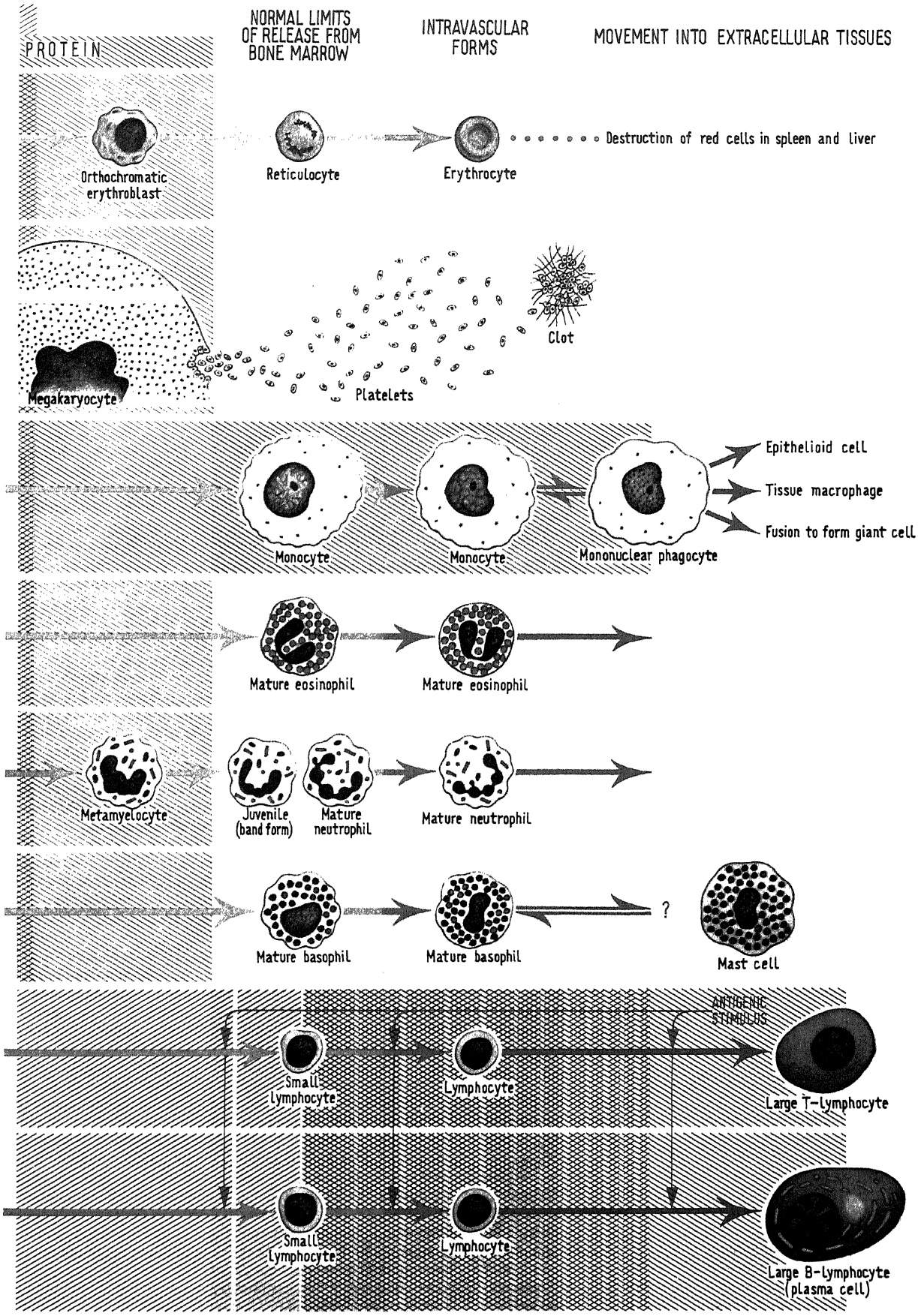
Haemopoietic tissue. Cords and islands of haematogenous cells consist of clusters of immature haemal cells in various stages of development, typically several different cell lines being represented at each focal group. One or more macrophages of a dendritic shape lie at the core of each such group of cells, and may contain the iron-bearing molecules ferritin and haemosiderin. Besides the phagocytic functions already mentioned, there is evidence that such macrophages are important in transferring iron to developing erythroblasts for haemoglobin synthesis and may indeed exert control



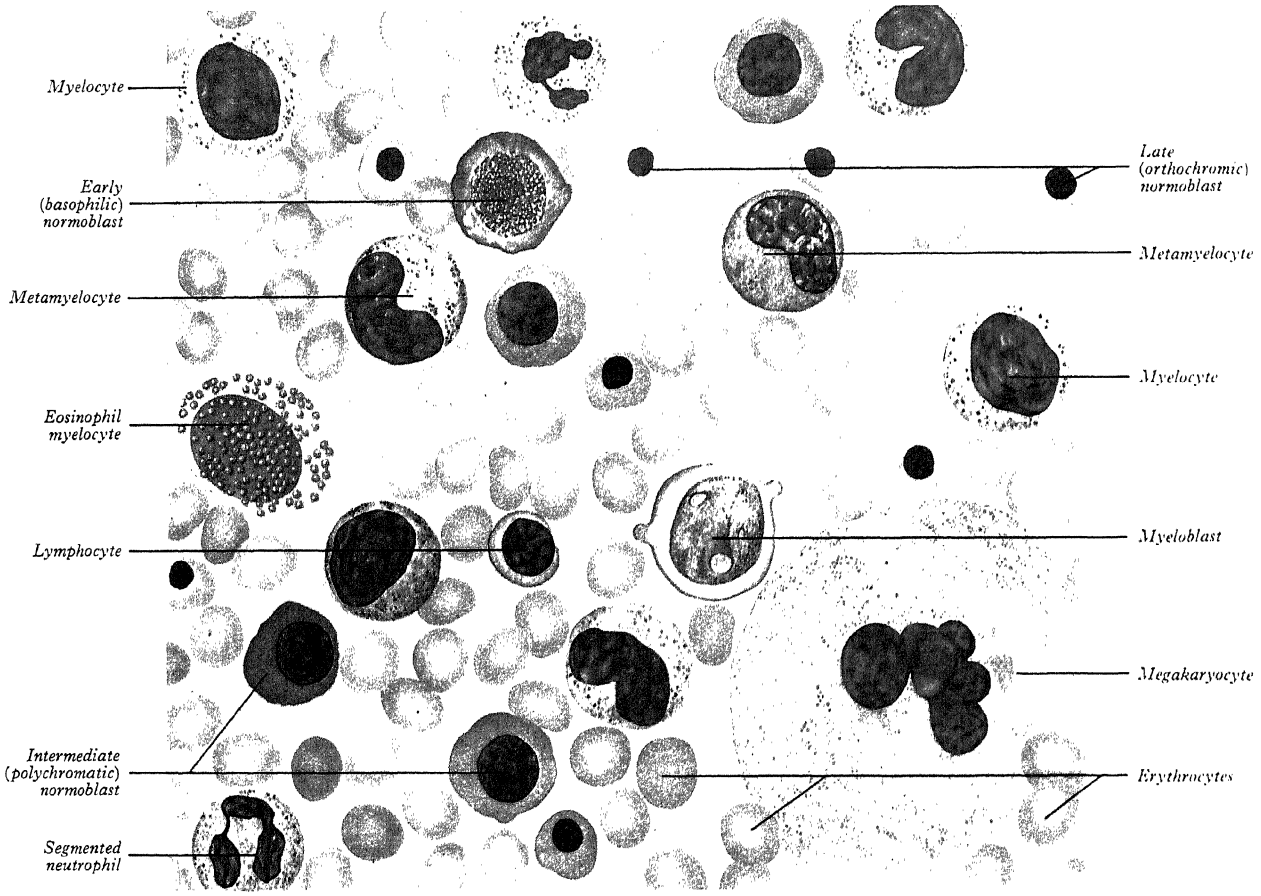
9.11 Diagram illustrating current views on haemopoietic cell lineages.



9.12 Haemopoiesis: a schema of the origins, developmental stages and fates of the different classes of haemal cells. Where details of development are uncertain, putative routes are shown in broken lines. Developmental pathways of the prenatal period are indicated (left) in red and different



stages of cellular development are hatched diagonally according to the column headings. It should be noted also that there is evidence for macrophage proliferation in the extravascular tissues, a finding not indicated in this scheme.



9.13 Smear preparation of normal human red bone marrow, obtained by sternal puncture. (This is a composite figure, drawn from a number of normal smears prepared by the late R L Waterfield, Guy's Hospital, and stained with a modification of Leishman's stain.)

over the rate of cell proliferation and maturation of the neighbouring haemopoietic cells.

Vascular supply to the bone marrow

No lymph vessels have yet been demonstrated in either yellow or red marrow, as might be expected in a system of spaces enclosed in a rigid casing of bone. The vascular supply is derived from the nutrient artery to the bone and drained by the accompanying vein (p. 469). The artery ramifies in the bone marrow, its branches terminating in thin-walled arterioles from which an extensive plexus of irregularly shaped sinusoids arises. These, in turn, drain into disproportionately large veins (Brookes & Harrison 1957). Many of these sinusoids are collapsed at any instant and are frequently but erroneously referred to as intersinusoidal capillaries.

HAEMAL CELL FORMATION (9.10–13)

The initial stages of differentiation of haemal cells in mature marrow are far from clear. Early attempts to trace cell lineages in bone marrow preparations solely by histological examination of normal and pathological tissue led to much controversy about the origins and relationships of the various types of cell which could be observed. Some investigators considered it likely that all cell lines arose from a single type of stem cell (*haemocytoblast*) in adult tissue (the *monophyletic theory* of Maximow and others), whereas other authorities favoured a multiple origin from many different types of stem cells (the *polyphyletic theory*, propounded by Ferrata and colleagues). Yet others have suggested compromise solutions. For many years it was also assumed that the lymphocytes arose exclusively in the peripheral lymphoid system and not in the bone marrow.

More recently, many experimental methods have been devised in an attempt to settle this issue; these include cell and organ culture, the replacement or transfusion of radiation-inactivated bone marrow with genetically or radioactively labelled haemal cells and the separation of stem cells from peripheral blood. The picture emerging still presents uncertainties and applies mainly to rodent experimental models rather than directly to man, although the analysis of human cell lineages is a major area of research because of the need for marrow transplantation for the remedy of genetic diseases and neoplastic pathologies.

Haemopoiesis in embryonic life. This commences at about the third week of gestation in the mesenchymal *blood islands* of the yolk sac, where only giant nucleated erythroid cells, *megaloblasts*, are formed. But these are soon replaced by *nucleated fetal erythroblasts* and these, in turn, are replaced by moderately large *anucleate, biconcave fetal erythrocytes* by about the fourth month of gestation. The adult type of erythrocytes is present by full term. Megakaryocytes, then granulocytes, lymphocytes and monocytes appear in that order between the second and fourth months of gestation. During the earlier developmental stages, stem cells occur in the yolk sac, liver, spleen and early bone marrow (9.8) and these tissues can give rise to **all** *haemal cell lines*, including lymphocytic ones, if they are transplanted to adult spleens (i.e. they are haemally 'totipotent'). Meanwhile, the lymphoid organs start to be colonized by stem cells which under normal circumstances only give rise to lymphocytes and hence appear to be already committed to that particular line of cells. However, the ability of these lymphoid tissues to carry out full haemopoiesis under certain pathological conditions suggests that some uncommitted stem cells have been retained in these sites but are normally suppressed.

Haemopoietic stem cells

The cell lineages of haemopoietic tissue have not yet been entirely

worked out, and there are a number of uncertainties, especially about the relation between the various stem cells. At the present time, the evidence suggests that within the adult marrow there is a very small number of *totipotent stem cells* capable of giving rise to all haemal cell types including lymphocytes. These differ from all other stem cells in one other respect, their ability to replicate themselves as well as produce lineage cells (therefore sometimes called *self-replicating stem cells*, SRTC, or *totipotent haemal stem cells*, THSC). The offspring of these cells, as well as maintaining the totipotent population, can give rise to lymphocytic stem cells of the T or B lines, or differentiate into pluripotent haemal stem cells which then divide into a series of stem cells for the different blood cells. The pluripotent haemal stem cell corresponds to the *colony forming unit stem cell* (CFU-S) of rodents, and its offspring to the precursors of each cell line, that is for the erythrocytic series, *burst forming units of the erythroid line* (BFU-E) for the monocyte/granulocyte line, *colony forming unit—granulocytes and macrophages* (CFU-G/M) giving rise to monocytes (and hence macrophages), eosinophils and possibly basophils; and for the megakaryocyte platelet line, *colony forming unit—megakarocyte* (CFU-Mk). Further differentiation along these lines is described below (see also **9.12**).

To generate a complete set of blood cells from a single totipotent cell takes a considerable period, up to some months, whereas the time is much shorter for the later stem cells to form their particular lineages, although because they are not self-renewing, grafts from these eventually fail as the cells they produce eventually age and die. This is of considerable importance in bone marrow replacement therapy, since the presence of totipotent stem cells in the donor's marrow is essential for success.

Next, we turn to the problem of the differentiation of such stem cells into the various cell lines of the blood. The early stages of these lines are difficult to distinguish since usually they cannot be recognized until well after they have begun to differentiate. In the cell line, however, a definite sequence of major stages can be recognized (or ascribed) (see **9.11, 12**). These stages are:

- the *final commitment* of a stem cell to a particular line of differentiation
- early cell *proliferation* to form a large pool of dividing cells
- *differentiation* as specific proteins characterizing the particular line are synthesized, accompanied by the gradual cessation of cell division
- final *maturation*, marked by the gradual closure of protein synthesis
- *release* of the cells from the bone marrow parenchyma into the circulation by passage through the endothelial linings of the sinuses.

In the case of some cell lines, cell division and protein synthesis may continue outside the bone marrow (e.g. monocytes or macrophages) and maturation may also be completed after release (e.g. erythrocytes).

The earliest 'committed' stages of each cell line are outwardly similar to the CFU-S, but at the next proliferative stage differences begin to emerge. In all, however, the initial picture is that of a rapidly dividing, relatively undifferentiated cell, in which the nucleus is large and euchromatic, with prominent nucleoli; the moderately basophilic cytoplasm contains unattached polyribosomes and the total cell size is large. As proliferation proceeds, the size of the cell usually diminishes as cell division outstrips cell growth. With continuing differentiation the ribosomes become numerous and the cytoplasmic basophilia increases as the specific messenger (m)RNAs begin to be synthesized, whilst the nucleus gradually becomes more heterochromatic and smaller, as DNA synthesis ceases; ultimately the nucleus becomes multilobed or pyknotic as protein synthesis terminates. The completed cell is then ready to be released, although the precise timing of its passage from the bone marrow varies with metabolic conditions and the 'demand' for more cells, so that relatively immature types of cell may be found in the circulating blood under abnormal conditions. This 'shift to the left' (a graphic convention of haematologists in which the cell is represented as maturing from the left to the right) is a useful concept in diagnostic haematology. These general features of development can be seen in several lines of haemal cells and underlie what appears at first sight to be a highly divergent series of progressions.

Erythropoiesis (9.11–13)

The earliest erythroid progenitor cells have not yet been identified, but the second stage includes cells which, after some delay, can multiply very rapidly to form numerous erythroblast cells; they have been named *burst-forming units of the erythroid line* BFU-E. Third in this lineage is a cell which is sensitive to the hormone erythropoietin, which induces it to further differentiation along the erythroid line. This is the *erythropoietin-dependent colony forming unit* (CFU-E).

The first readily identifiable cell of the erythroid series is the *proerythroblast* (*pronormoblast*), a large (14–20 μm) cell with a large euchromatic nucleus and moderately basophilic cytoplasm. The latter already has small amounts of ferritin and bears some of the protein spectrin attached to its plasma membrane (p. 1401); both are characteristic of this cell line and can be detected by electron microscopy. Proerythroblasts proliferate, and haemoglobin-RNA synthesis begins, as the smaller (12–17 μm) *basophilic* or *early erythroblast* (*basophilic normoblast*) appears, rich in ribosomes. Shortly afterwards, haemoglobin synthesis commences so that the cytoplasm becomes partially eosinophilic (the *polychromatophilic* or *intermediate erythroblast/normoblast*) which has a diameter of 8–12 μm. At this stage, most of the cytoplasmic RNA is lost and the nucleus, becoming intensely pyknotic, is finally extruded from the cell, thus leaving an anucleate *reticulocyte*. At this point the cell is released into the circulation, losing its residual RNA in a few days to become a mature *erythrocyte*. The nucleus is phagocytosed by a macrophage. The whole process of erythropoiesis takes 5–9 days; after release, reticulocytes typically sojourn for up to 2 days in the marrow sinusoids and then for an equal time in the spleen, perhaps because of their particularly adhesive cell coat. During this period, the remaining ribosomes add a further small amount of haemoglobin to the cell; then all the organelles are finely dismantled by a soluble cytoplasmic enzyme system involving the protein ubiquitin.

The cell lineage of normal erythrocytes is often called the *normoblastic series* to distinguish it from abnormal erythroid lines; too few divisions of the early proliferative stages may give rise to abnormally large erythrocytes (*macrocytes*), whereas too many divisions, or insufficient early growth, can lead to the formation of abnormally small *microcytes*. Disturbances in haemoglobin synthesis can also give rise to a variety of anaemias and various other pathologies.

Granulocytopoesis (9.11–13)

The details of the processes by which the granulocytes are formed are best known for the *neutrophil*, which will be described in some detail. Initially, the putative stem cells transform into the large (10–20 μm) *myeloblasts* which are similar in general size and appearance, though not in internal details, to the proerythroblast (see above). These proliferative cells differentiate into the larger *promyelocytes*, in which the first group of specific proteins is synthesized in the granular endoplasmic reticulum and Golgi apparatus, both of these organelles being quite prominent. The proteins are stored in large (0.3 μm) *primary* ('*non-specific*') *granules*, large lysosomes containing acid phosphatase and having azurophilic staining properties (p. 1402). Next, in the smaller *myelocyte*—the last proliferative stage—the smaller *secondary* ('*specific*') *granules*, which contain a slightly different enzyme array, are formed in a similar manner, though described as being released into the cytoplasm from the '*cis*' side of the Golgi body, whereas the primary granules arise from the other ('*trans*') side (see p. 31). The nucleus is typically flattened on one side in myelocytes. Subsequently, in the *metamyelocyte* stage the cell size decreases further, the nucleus becomes heterochromatic and horseshoe shaped and protein synthesis practically ceases. Finally, as the neutrophil is released, the nucleus becomes heavily indented (the *juvenile* or '*stab*' form) and then partially divided into up to six lobes (the *segmented* or *mature neutrophil*). The whole process takes about 7 days to complete: the mitotic period about 3 days and maturation 4 days. They may then be stored in the medulla for a further 4 days, depending on demand, before final release into the circulation.

Eosinophils pass through a similar sequence except that their nuclei never become as irregular as that of the neutrophil, and only one set of lysosomal granules is synthesized. It is not certain whether the eosinophil differentiates from the same myeloblast (or promyelocyte)

stock as the neutrophil, or whether it is distinct from the colony forming unit (CFU-G/M) stage, which at present seems more likely. In the case of *basophils*, it is not certain that they follow this general sequence at all; they may not even share this CFU as an ancestor.

Monocyte formation (9.11, 12)

Monocytes are also formed in the bone marrow. Monocytes and neutrophils appear to be closely related cells and arise from the same stem cell, the *colony forming unit for granulocytes and macrophages* (CFU-G/M) (the granulocytes in question being, of course, neutrophils). Subsequently they pass through a proliferative *monoblast* stage and then form differentiating *promonocytes* in which small lysosomes begin to be made (these may be demonstrated by neutral red staining). After further divisions, monocytes are released into the general circulation, and at least some are believed to pass to perivascular and extravascular sites, which they then populate as *mononuclear phagocytes* or *macrophages*.

Thrombocytopoiesis (9.11, 12)

Platelets, being fragments of cells, arise in a most unusual manner by the division of the cytoplasm of certain huge cells into many portions (Pennington 1979). The first detectable cell of this line is the highly *basophilic megakaryoblast* (15–50 μm); this is followed by a *promegakaryocyte* stage (20–80 μm) in which synthesis of granules begins; finally, the fully differentiated *megakaryocyte*, a giant cell (35–160 μm) with a large, dense, *polyploid*, *multilobate* nucleus, emerges. Once differentiation has commenced, mitoses proceed without cytoplasmic division and the chromosomes are retained within a single polyploid nucleus containing 8n, 16n or 32n chromosomes, depending upon how many nuclear mitoses finally occur. Under the electron microscope, the cytoplasm is distinguished by numerous centrioles and spindle microtubules, both of which reflect the repetitive mitotic activity. Meanwhile, differentiation proceeds in the cytoplasm with the production of free polysomes, smooth endoplasmic reticulum and fine basophilic granules. Cytomembranes within the cell fuse with one another and with invaginations of the plasma membrane to cut off portions of cytoplasm, which then break away from the parent cell to form platelets. The nucleus of the megakaryocyte eventually disintegrates. The release of platelets into the circulation has been described as involving first the protrusion of a long, narrow extension of the megakaryocyte cytoplasm through an aperture in the sinusoidal epithelium, which then separates at its end into individual platelets.

Control of haemopoiesis

The numbers of cells in the circulation are closely regulated in adult life, cell destruction being counterbalanced by cell replacement. How this system of control operates is known, at least in part, only for erythrocytes. Erythropoiesis is stimulated by a circulating protein *erythropoietin* synthesized by the tissues of the kidney and other parts of the body. The rate of erythropoietin synthesis is inversely proportional to the oxygen content of the tissues; hence low oxygen tensions, usually consequent upon lowered erythrocyte numbers, stimulate erythropoiesis whereas high oxygen tensions cause the withdrawal of the stimulus. At high altitude the lowering of the partial pressure of oxygen in the atmosphere leads to a raised erythrocyte count.

Many other factors also affect the rate of haemopoiesis, for example thyroid hormones, somatotrophic hormone androgenic steroids and other hormones. In recent years it has also become clear that many other factors affect haemopoiesis, including cytokines and growth factors released by T lymphocytes, macrophages, neutrophils and other cells. The numbers of cells in the blood show a *diurnal rhythm*, probably because of hormonal fluctuations. Infection, haemorrhage and other clinical disturbances also affect the pattern of cell production, as do cytotoxic chemicals and ionizing radiations, to which the dividing cells of the bone marrow are particularly susceptible.

MONONUCLEAR PHAGOCYTE SYSTEM

As noted above, the body deploys a range of defensive cells to perform many complex co-ordinated activities vital to survival in the face of immense pressure of attack from potentially pathogenic organisms. These cells include lymphocytes, to be considered later, and a range of phagocytes capable of ingesting and destroying micro-organisms. The phagocytic roles of neutrophil leucocytes have already been described (p. 1402). The other major types of phagocyte comprise a family of cells with related lineages and various molecular features in common, present in the vascular and extravascular tissues throughout the body. They constitute the *mononuclear phagocyte system*. Its members include the blood *monocytes*, *macrophages* of various kinds in extravascular tissues, and some mildly *phagocytic cells* which are extremely effective at stimulating immune responses in lymphocytes (antigen-presenting cells). They at all times express a characteristic group of surface molecules, the Class II major histocompatability complex (MHC) molecules (p. 1420) which enable them to select and stimulate lymphocytes appropriate to combating specific antigens. Recently it has become obvious that various other cells outside this system can also express MHC II molecules when suitably stimulated, but the mononuclear phagocytes appear to be the only cells to be permanently in this state. The term 'mononuclear phagocyte system' was originally proposed by Aschoff in 1924 to embrace different classes of phagocytic cells, most of which we now know to be macrophages, but this title has been extended more recently to cover various other related cells, as noted above. It is also in part equivalent to the *reticulo-endothelial system* of earlier times (a term that is still occasionally used, although generally superseded).

Although the phagocytic and antigen-presenting abilities of macrophages and related cells are of major importance, it has been discovered that these cells also engage in the synthesis and secretion of many *cytokines*—soluble proteins which regulate highly diverse aspects of cell biology such as growth, mitotic division and differentiation, tissue repair and modelling, as well as defence.

Mononuclear phagocytes can be divided into two groups: the macrophages, which are highly phagocytic ('professional phagocytes'), and the other antigen-presenting cells concerned primarily with lymphocyte activation.

MACROPHAGES

As a historical accident, the cells of this system were originally given different names according to their location. In connective tissue they are either *fixed* or *wandering macrophages* (histiocytes, clasmatocytes); in the liver they are called *littoral cells* of the sinusoids (von Kupffer cells); in the central nervous system, *microglial cells*; and in the meninges, *meningocytes*. They are known as *pleural*, *peritoneal*, *alveolar* and *splenic macrophages* in the sites denoted by these names, and in the synovial joints they are types *A* and *B* *synovial cells* (p. 497). In the blood they are represented by *monocytes*. In subserous tissue of the pleura and peritoneum, macrophages often aggregate as *milky spots*, near small lymphatic trunks. In the spleen they also occur in clusters (ellipsoids) around the exits of small (pencillar) arterioles (p. 1441) and diffusely throughout the splenic cords, while in the haemopoietic tissue of the bone marrow they are intimately associated with differentiating haemal cells (stromal macrophages).

Macrophages vary in structure depending on their location in the body. All are large cells (15–25 μm across) with a moderately basophilic cytoplasm containing some granular and agranular endoplasmic reticulum, an active Golgi complex, mitochondria, etc. and a large, euchromatic nucleus, signifying an active metabolism and continuing synthesis of lysosomal enzymes (unlike neutrophil granulocytes which virtually cease synthetic activity shortly after leaving the bone marrow). All cells have irregular surfaces with many filopodia and contain varying numbers of endocytic vesicles and lysosomes. Some macrophages are highly motile, whereas others tend to remain attached and sedentary (e.g. the littoral cells of hepatic and lymphoid sinuses).

Within the connective tissue, macrophages may fuse to form large syncytia (foreign body giant cells) around large particles which are too big to be phagocytosed, or when stimulated by the presence of infectious organisms, for example tubercle bacilli (epithelioid cells). As mentioned above, all of these cells express Class II MHC molecules on their surfaces; they are also positive for the characteristic (though not specific) molecular surface marker International

Cluster Determinant (CD)14 and the specific cytoplasmic granules marker CD68. Macrophages in certain localities express additional markers, for example CD33 in myeloid tissue and CD16 in pulmonary alveoli. Microglia also possess CD4 on their surfaces, as do T helper lymphocytes, a feature which unfortunately makes them susceptible to human immunodeficiency virus (HIV) infections, too.

Origins of macrophage cell lineages

There is now much evidence that macrophages arise in the bone marrow from stem cells concerned with the production of the neutrophil granulocyte-monocyte lineage (the colony forming unit—granulocytes and macrophages; CFU-G/M stem cell, see p. 1414), monocytes mostly representing blood-borne macrophages en route to their final extravascular tissue destinations. When the cells enter these tissues through the endothelial walls of capillaries and venules, they can undergo a limited number of rounds of mitosis before they die and are replaced from the bone marrow, although alveolar macrophages of the lung appear to be able to undergo many more mitotic divisions than those elsewhere. Some cells may remain quiescent for long periods of time, for example microglial cells in the central nervous system. Their mature morphology and activities appear to be largely determined by the tissues in which they reside, and cells appear to be seeded fairly randomly from the bone marrow.

Osteoclasts and chondroclasts may also be in some distant way related to these cells, since they have several structural and functional similarities and also arise from stem cells in the bone marrow; however, for various reasons including the existence of a number of distinctive molecular markers, it appears unlikely that they originate directly from the monocyte lineage (p. 459).

Macrophage functions

Macrophage functions include: phagocytosis, antigen presentation, cell and tissue regulation and remodelling in growth and repair, secretion of cytokines, and anti-tumour activity amongst a wide repertoire. In particular they are intimately involved in the mechanisms of innate and acquired immunity (see, for example, Lewis & McGee 1992; Gordon et al 1992).

Phagocytosis. The uptake of particulate material and organisms is carried out by macrophages in many locations. In general connective tissue they can dispatch invading micro-organisms and remove debris engendered by tissue damage, or engulf apoptotic cells in differentiating or remodelling tissue. In the lung, alveolar macrophages constantly patrol the surfaces of the air-filled cavities, into which they migrate from pulmonary connective tissue; there they engulf inhaled particles including bacteria, surfactant and debris and many enter the sputum (hence 'dust cells' and, in cardiac disease, 'heart failure cells' full of extravasated erythrocytes). Similar scavenger functions are performed in the pleural and peritoneal cavities. In lymph nodes they line the walls of sinusoids ('littoral macrophages') and remove particulate matter from the passing lymph as it percolates through their narrow sinuses. In the spleen and liver, macrophages carry out similar acts of particle removal but here they are also involved in the detection and destruction of aged or damaged erythrocytes, whose haemoglobin they begin to degrade preliminary to recycling iron and amino acids (see p. 1402). The phagocytic activities are greatly increased when the target has already been coated in antibody or complement (or both), since macrophages have Fraction crystalline (Fc) and complement component receptors on their surfaces, initiating endocytosis. Once phagocytosis has occurred, the vacuole bearing the ingested particle fuses with endosomal vesicles containing a wide range of lysosomal enzymes (over 100 have been described), including many hydrolases, and oxidative systems capable of rapid bacteriocidal action. These activities are much enhanced when macrophages are stimulated by various cytokines (*activated macrophages*), such as Interferon (IFN)-γ, secreted by other cells of the immune system, especially T lymphocytes. Lysosomal enzymes may also be secreted from the macrophage on to target microbial cells as part of the defensive mechanism.

Antigen presentation. The part which mononuclear phagocytes play in immune responses is complex and incompletely known. As mentioned elsewhere (p. 1420), they bear Class II MHC antigens at their surface, which enable them to stimulate different classes of T lymphocyte through close-range interactions: macrophages phagocytose alien antigens and partially digest them in their endosomal systems, passing some of the antigenic remnants to the cell surface where they are bound by MHC molecules. This complex of alien antigen and MHC molecule is then presented to a T lymphocyte, which, if it possesses the appropriate receptor on its surface, is stimulated in various ways, depending on the type of T cell involved (p. 420). In turn, macrophages are also affected by activated T and B lymphocytes; and cytokines (including macrophage-activating factors, Interleukin II, etc.) secreted by certain T cells can determine their migration and degree of phagocytic activity (p. 1420). Under such influences the macrophages themselves can synthesize and secrete various other bioactive substances, for example Interleukin I which stimulates the proliferation and maturation of other lymphocytes, greatly amplifying the reaction of the immune system to foreign antigens. When activated by Interleukin II and other cytokines, macrophages synthesize and release many other bioactive molecules, including Tumour Necrosis Factor (TNF)-α which is able to kill neoplastic cells and appears to be a mechanism for the removal of small tumours which may appear in the body from time to time throughout life; it is interesting that TNF-α also depresses the anabolic activities of many cells in the body and this may be a major factor in cachexia (wasting) which typically accompanies more advanced cancers. Other macrophage products include plasminogen activator, promoting clot removal (p. 1406), and various lysosomal enzymes, several complement and clotting factors and lysozyme (an antibacterial protein). In pathogenesis, such substances may be 'erroneously' released to damage healthy tissues, for example in rheumatoid arthritis and various other inflammatory conditions. As noted above, because macrophages have receptors on their surfaces for the Fc ends of antibodies and for the C3 component of complement, and so can bind readily to and avidly phagocytose antibody-coated (opsonized) microbes and other 'alien' material. Such close antibody-mediated binding may also initiate the release of lysosomal enzymes on to the surfaces of the cellular targets to which the macrophages are bound (an example of Antibody-Dependent Cell-Mediated Cytotoxicity, ADCC, also shown by various other cells including neutrophils), particularly if these are too large to be phagocytosed (e.g. nematode worm parasites such as *Wuchereria bancrofti*). These reactions are much enhanced when macrophages are stimulated by cytokines.

Macrophages and related cells also produce several growth and differentiation factors with actions on other tissues. They release factors which stimulate haemopoiesis, including erythropoietin, and have complex metabolic actions through their production of prostaglandins, thromboxanes and other bioactive substances, including the stimulation of bone resorption by osteoclasts.

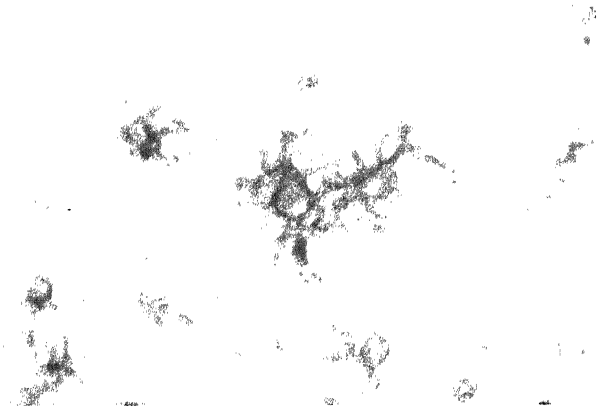
In summary, these various activities appear to be related to three quite distinct roles: the first associated with defence, the second with repair and regeneration of damaged tissues and the third with the ongoing maintenance of normal cell proliferation and differentiation in healthy tissues throughout the body. The full extent of macrophage-mediated effects has yet to be determined and some of them are quite unexpected; for example high levels of Interleukin I induce sleep, as found in some severe systemic infections. It seems that macrophages may be intricately connected with a vast array of cellular activities in a network of great complexity.

ANTIGEN-PRESENTING CELLS (9.14, 15)

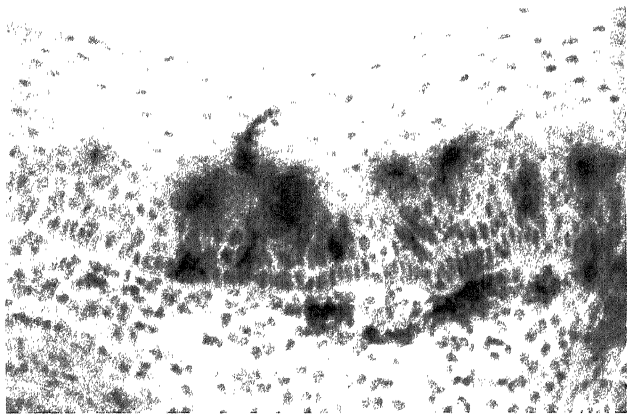
Whilst macrophages are able to present antigens to lymphocytes, there is a category of mononuclear phagocytes which are perhaps a thousand times more effective at performing this function. These are all cells with a highly folded surface, often elongated into dendrites. They include the *interdigitating cells* and *follicular dendritic cells* of secondary lymphoid tissue, *Langerhans cells* of the epidermis (see, for example, Knight & Stagg 1993) and microfold and reticulated cells of the alimentary epithelium. These cells are mildly phagocytic, so that they are able to endocytose alien antigens, partially digest them, then present them on the surface in combination with Class II MHC molecules. Here they can bind to receptor molecules on the surfaces of lymphocytes to select and stimulate appropriate clones of these cells.

Interdigitating cells

Interdigitating cells are found in T-cell rich areas of secondary

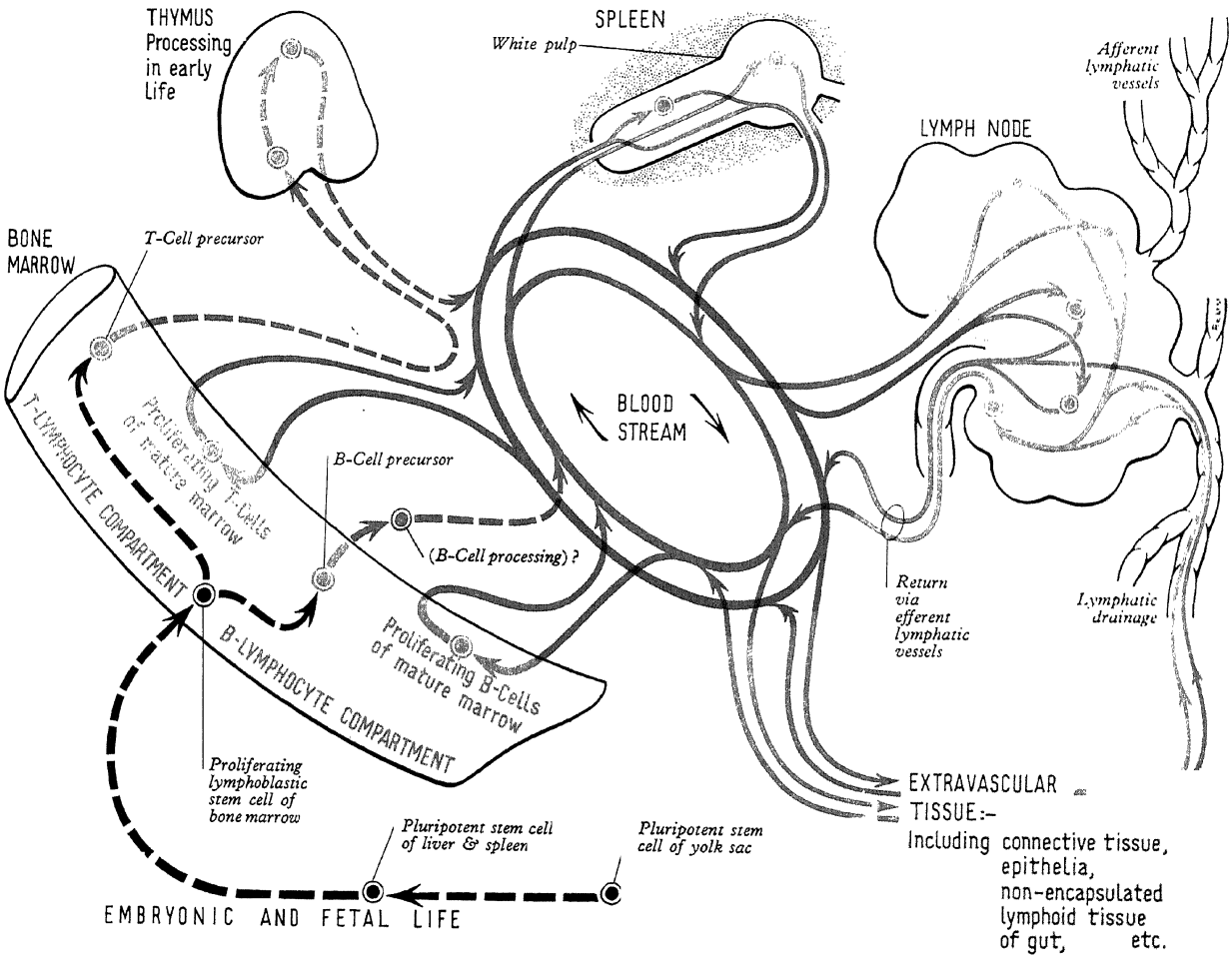


9.14 Follicular dendritic cells in the germinal centre of a palatine tonsil, (immunoperoxidase method). (Provided by M Perry, Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)



9.15 Langerhans' cells, in the epithelium overlying a palatine tonsil. Immunoperoxidase method. (Provided by M Perry, Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)

lymphoid tissue (paracortical areas of lymph nodes, interfollicular areas of mucosa-associated lymphoid tissue (**9.52**), periarteriolar sheaths of splenic white pulp). The MHC II-antigen complexes on their surfaces bind specifically to the receptors on T-cell surfaces (T-cell receptors; TCR) accompanied by other adhesive and secretory interactions between these two cell types. This activates T cells to proliferate and prime them prior to carrying out their immunological activities. Only T cells with receptors corresponding to the specific antigen presented to them in combination with the MHC II molecules can be triggered in this way. The process is hence termed Class II MHC restriction.



9.16 Diagram depicting the current views of the origins and circulation of the two major classes of lymphocyte from the bone marrow to the peripheral lymphoid tissues. Red = B lymphocytes; blue = T lymphocytes.

Follicular dendritic cells (9.15)

Follicular dendritic cells are similar cells, with long dendritic extensions, found in the follicles of secondary lymphoid tissues where they carry out similar antigen presentation to B cells. Only those B cells which possess appropriate receptors on their surfaces can be stimulated to divide and secrete antibody by this contact, so again these cells are part of the highly selective mechanism by which suitable immune responses are made to specific antigens.

Langerhans' cells

Langerhans' cells are similar dendritic cells present in the epidermis (5.11); they contain characteristic elongated membranous vesicles (Birbeck granules) of uncertain function, and appear to be involved in the immune responses of lymphocytes within the skin to antigens within the epidermis. Such cells (with the same curious granules) have also been found in lymph nodes and the thymic medulla, and it has been suggested that Langerhans' cells may migrate into these structures after they have picked up antigens, to stimulate the lymphocytes within them. The lymphocytes are then envisaged as passing back to the skin to carry out defensive actions there, or to populate regional lymph nodes to deal with any infectious agents which might pass into them from the lymphatic drainage of the area.

Reticulo-endothelial system

The concept of an interrelated system of mononuclear phagocytes has in recent years supplanted that of the reticulo-endothelial system, which at its inception was envisaged as a diffuse network of various phagocytic cells, mainly endothelial, lying in or in close proximity to the walls of certain blood vessels and lymphatics. It was found that these cells took up many particulate dyes and other substances infused into blood and lymph and thus seemed well fitted for the removal and destruction of particulate materials from these fluids. However, more detailed study showed that, in general, true endothelial cells are only mildly phagocytic, as indeed are many other tissue cells; the highly phagocytic cells are not endothelial cells at all, but types of macrophages of myeloid origin and, unlike endothelial cells, bear Class II MHC molecules at their surfaces.

As stated at the beginning of this section, the defence of the body against pathogens depends on the concerted efforts of a wide variety of cell types, present in vast numbers. The cells of the blood and mononuclear phagocytes can attack the alien organisms directly by phagocytosing them or liberating enzymes or other toxic substance on to their surfaces. In the lymphoid system, a battery of other defensive measures is provided against pathogenic organisms by several classes of lymphocytes. These have some remarkable properties: some of them (B lymphocytes or B cells) synthesize and secrete antibodies which can specifically recognize and neutralize a huge range of alien macromolecules (antigens) and prime various non-lymphocytic cells to engage in phagocytosis and other defensive manoeuvres. Other (T lymphocytes) can recognize and selectively kill virus-infected cells, or modulate the activities of other lymphocytes and phagocytes. Lymphocytes proliferate, differentiate and mature in specialized *lymphoid tissues*, collections of such cells and related antigen-presenting cells (APCs) which are situated in many sites within the body. Before considering these, the biology of the lymphocytes themselves will be briefly reviewed.

ORIGINS OF LYMPHOCYTES

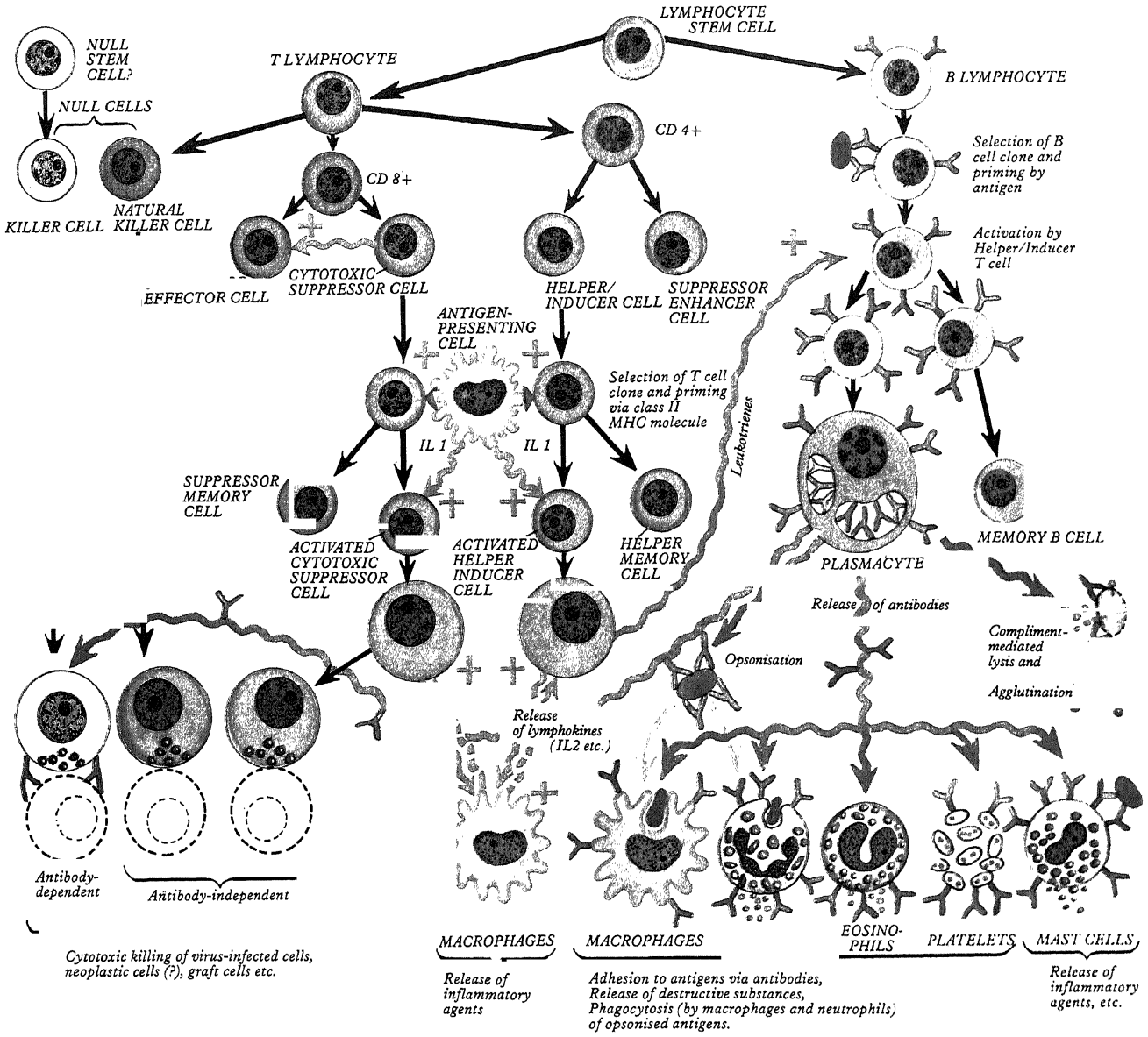
Much of the knowledge of lymphocyte life history (9.16, 17) has come from experimental situations which include the tracing of radioactivity or genetically labelled cells, the latter involving transfusion of lymphocytes with chromosomal abnormalities into normal but inbred strains of laboratory animals. Other experiments have been based on the sensitivity of lymphocytes to ionizing radiation, the various components of the lymphocytic system being eliminated by selective irradiation, accompanied by appropriate transfusions or transplants of lymphopoietic tissue from bone marrow, lymph nodes, thymus and so forth. Thirdly, selective surgical removal of various lymphopoietic components and the removal of lymphocytes from the lymphatic channels has been carried out. In addition to these manoeuvres, a whole battery of immunological techniques has been brought to bear on the problem, including immunoassay, tissue culture methods, and techniques for the localization of antibodies by their reactions with antigens or antigen–antibody complexes previously labelled with fluorescent compounds or electron-dense substances (or enzymes), with subsequent examination by fluorescent and electron microscopy respectively.

Lymphocytes originate in the embryo from mesenchymal cells in the yolk sac initially, and later in the liver and spleen. These primitive lymphoid *stem cells* subsequently take up residence in the bone marrow, which becomes the only site of stem cell proliferation after birth (however, see p. 1412). When these stem cells divide, they give rise to further stem cells and to lymphoblasts which continue to divide, eventually becoming small lymphocytes. Some of these pass in the blood circulation to the thymus where they migrate into its cortex and divide repeatedly, undergoing a selection process to determine if they will be suitable members of the immune system's repertoire (p. 1427); the resulting small thymus-processed T lymphocytes then enter the bloodstream and migrate to the peripheral (secondary) lymphoid tissues of lymph nodes, spleen, alimentary, respiratory and urogenital tracts (Mucosa-Associated Lymphoid Tissue, MALT), and bone marrow. They enter these through the walls of postcapillary venules. Within these centres the T lymphocytes migrate to specific areas: in lymph nodes, the paracortex between the cortex and medulla; in the spleen, the periarteriolar sheaths; in MALT and bone marrow, the areas between or neighbouring the lymphoid follicles. From these, the lymphocytes can enter the efferent lymphatic drainage, returning to the bloodstream via the thoracic and right lymphatic ducts and the brachiocephalic veins, and so eventually back to the lymphoid tissues again; in the spleen they may also migrate directly into the bloodstream. This *circulation of lymphocytes*, first established by Gowans, is responsible for the large number of T lymphocytes found in the blood (9.16). When stimulated antigenically such lymphocytes enlarge and multiply, and their progeny are capable of a number of different defensive actions concerned with the regulation of immune responses and the elimination of virus-infected and other potentially pathogenic cells (see also below).

The *B lymphocytes* do not pass through the thymus, but undergo a phase of differentiation similar to that of the T lymphocytes, within the bone marrow itself. In birds, B lymphocytes are derived from a specialized diverticulum of the cloaca, called the *bursa of Fabricius* (giving this type of lymphocyte its title, the *bursa equivalent* or *B* lymphocyte, although subsequently it appears that such a prefix can be even more appropriately applied to denote the bone marrow origin of the B-cell class in mammals). Selected B lymphocytes then leave the bone marrow and migrate to peripheral lymphoid sites where they undergo yet another round of cell selection, but this time the major stimulus comes from antigen within the lymph nodes, presented to them by specialized *antigen-presenting cells*. On antigenic stimulation they multiply to form *germinal centres*; such lymphocytes can, while still within the lymphoid tissues or after further migration, mature into the large pyroninophilic (i.e. RNA-rich) plasma cell series, which produce antibodies in their extensive rough endoplasmic reticulum and secrete it into the adjacent tissues.

FUNCTIONING OF THE LYMPHOCYTIC SYSTEM

Lymphocytes, together with the phagocytes of the mononuclear phagocyte system, are responsible for the defensive reactions of the



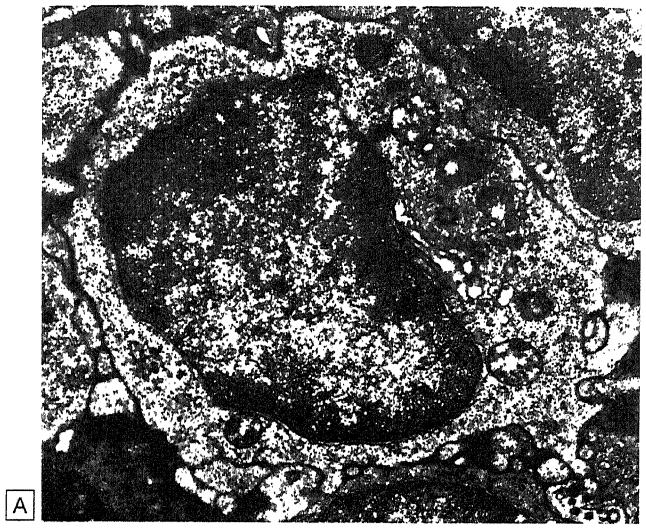
9.17 Schema of the origins and functional interactions by lymphocytes and other cells of the immune system. This is a highly simplified view of cellular interactions (see text for further and more detailed information).

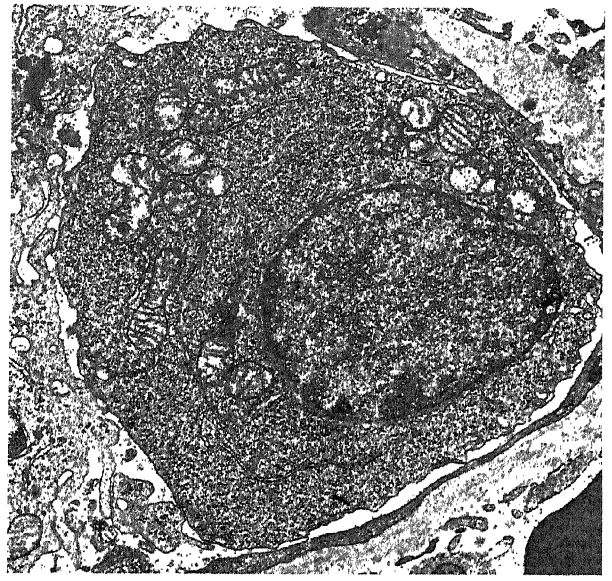
body, the former by non-phagocyte interactions of various kinds, the latter mainly by phagocytosis (**9.17**, 18). These defences are directed against alien chemical substances (antigens) which can vary in size and composition from a few amino acids to large and complex antigenic systems such as those of bacteria, viruses, fungi, protozoa, helminth worms, etc. or their harmful metabolites (toxins). They also play a part in the removal of any unwanted or abnormal materials within the body such as foreign or altered proteins and effete, neoplastic or virally transformed cells (auto-antigens or self-antigens).

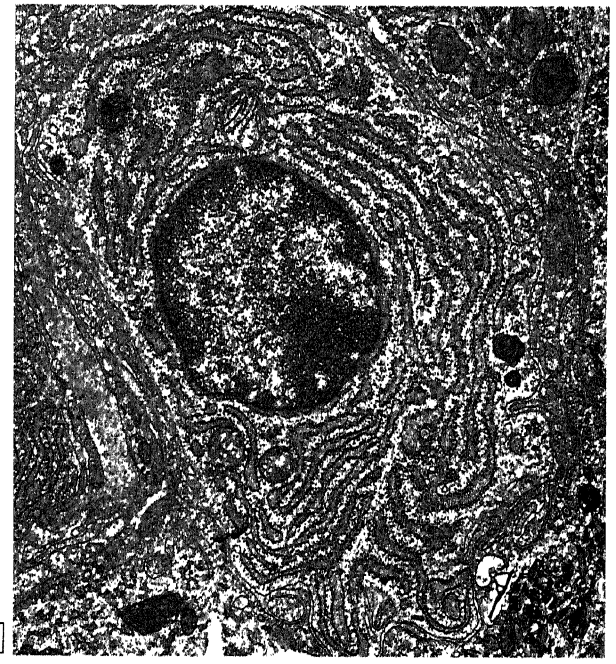
B lymphocytes (9.12, 17, 18A, B, D)

B lymphocytes can be antigenically stimulated by binding antigens via their B cell receptors (BCRs) after which they proliferate and their progeny transforms into larger B cells (*plasmacytes*) which synthesize and secrete *antibodies*; the latter chemically 'recognize' and bind specifically to their respective *antigens* to inactivate them or cause their destruction. Antibodies may circulate freely in the body fluids (*soluble antibodies*) or may be secondarily attached to a variety of defensive cells (*homocytotropic antibodies* or *cytophilic antibodies*) to enhance their activities or to enable them to carry out a wider range of functions.

Chemically, *antibodies* (or immunoglobulins) are proteins with a molecular mass of 150–950 kDa. Each antibody molecule consists of at least one or more basic units of four polypeptide chains (150 kDa), comprising two *heavy chains* and two *light chains* held together by disulphide links between the two heavy chains and between the heavy and light chains. The whole molecule can be thought of as Y-shaped, with the arms of the Y being able to swing around a central hinge region. These molecules are bifunctional, i.e. with two interactive poles. The N terminal regions of the molecules have highly variable (*V*) regions in their amino-acid sequences and are responsible for specifically binding to antigens via their Fab (Fragment antigen binding) ends. There are vast numbers of B-cell types within the body ($1–2 \times 10^{12}$ in normal adults), each one representing a different antibody specificity capable of binding to a specific antigen at its Fab end in a manner closely analogous to a 'lock and key' system. The other end of the molecule (the C terminus), is much less variable in structure and is known as the Fc end or the *constant region*, and it is this constant region that defines the antibody *class*. There are







9.18 Electron micrograph of lymphocytes. A A small lymphocyte. Magnification × 6000. (Provided by D R Turner, Guy's Hospital Medical School, London.) B Electron micrograph of a plasmacyte in loose connective tissue. Magnification × 6000. C Transmission electron micrograph of a stimulated T lymphocyte in the spleen (monkey). Note the numerous free ribosomes and relative paucity of granular endoplasmic reticulum. Magnification × 10 000. D Transmission electron micrograph of a stimulated B lymphocyte (plasmacyte) in the spleen (monkey). Note the copious granular endoplasmic reticulum containing newly synthesized antibodies, seen here as finely granular material. Magnification × 10 000.

five classes (*isotypes*) of antibodies distinguishable in the blood plasma and interstitial fluids. These are:

(1) Immunoglobulin *G* (IgG), which forms the bulk of circulating antibodies and is subdivided into four subclasses ($\gamma 1$, $\gamma 2$, $\gamma 3$ and $\gamma 4$);
(2) Immunoglobulin *M* (IgM), which is normally synthesized early in immune responses and is secreted as a pentamer, the monomers being joined near their Fc ends into a starlike aggregate by a small polypeptide called the *J* chain (15 kDa);
(3) Immunoglobulin *A* (IgA), which has two subclasses α_1 and α_2; IgA is present in secretions of the body, particularly saliva and other fluids of the alimentary tract as a monomer or polymer containing J chains and another peptide called the *secretory piece* (70 kDa) which is synthesized by epithelial cells of the mucous membranes (see p. 69);
(4) Immunoglobulin *E* (IgE), which is a *cytophilic* antibody, found on the surface of mast cells and the basophil granulocytes in the blood;
(5) Immunoglobulin *D* (IgD), of uncertain significance, found

together with IgM as a major membrane-bound immunoglobulin on mature immunocompetent B cells and thought to be important in the activation of these cells by antigen entering the immune system.

Certain tissue cells and leucocytes possess *isotype specific Fc receptors* for antibodies on their surfaces, conferring antigen-binding properties on these cells when they have bound antibodies, via their free Fab ends.

The defensive functions of antibodies are numerous. They can *agglutinate* antigens by forming cross-links between them, so rendering them inactive as infective agents (e.g. viruses and bacteria). After binding an antigen they may also activate the classical pathway of *complement* (a complex of at least 12 proteins in the plasma), which undergo a series of amplificatory reactions in a cascade manner similar to that of the blood coagulation system, finally causing the lysis of bacteria and other cells bound by antibody. If only parts of the complement proteins complex are bound (e.g. C3b), these can form bridges between antibody-coated target cells and phagocytic cells such as macrophages bearing C3b receptors on their surfaces, inducing their phagocytosis and ultimate destruction. Free antibodies may also bind to foreign antigens and then attach via their Fc ends to receptors on various defensive (effector) cells, which are then triggered to ingest or enzymatically damage the foreign antigens (*Fc receptor mediated endocytosis*); examples of such effector cells are macrophages and neutrophils and antibodies coating the antigens prior to endocytosis are termed *opsonizing antibodies* or *opsonins*. *Cytophilic antibodies* (i.e. those binding effector cells) may cause the activation of cells in other ways when they bind antigens. Thus, when IgE antibodies bound to mast cells encounter appropriate antigens, they trigger the release of histamine and other vasoactive agents (e.g. platelet-activating factors, leukotrienes, prostaglandins, etc.). This is an important mechanism in the host's defence against microbial invasion, but is also seen in an exaggerated form (hypersensitivity) in certain types of allergy, for example to the proteins of pollen, causing hay fever, asthma and other disorders.

B lymphocytes, themselves, can be activated to divide and differentiate into antibody-secreting *plasmacytes* by antigen–antibody complexes binding to Fc receptors on their surfaces. Activated T lymphocytes can also express Fc receptors and be induced to proliferate and secrete cytokines by this type of antibody-mediated mechanism. It should be emphasized that the antibody bound to these B and T lymphocytes or other cell types bearing the appropriate Fc receptors is derived from plasmacytes after passing into the tissue fluids or blood. Following antigen stimulation, some B lymphocytes are retained as a very long-lived pool of *memory* cells which are capable of responding to their specific antigens with a more rapid and higher antibody output and increased antibody affinity (a measure of the tightness of binding to antigen) compared with the primary response.

When circulating antibodies bind to antigens they form *immune complexes* which, if present in abnormal quantities, may cause pathological damage to the vascular system and other tissues, either, as in some types of glomerulonephritis, by interfering mechanically with membrane permeability to fluids, or alternatively, by causing local activation of the complement system to attack cell membranes, thus causing vascular disease. In pregnancy some maternal IgG is transferred across the placenta, conferring *passive immunity* on the fetus; but in the case of Rhesus factor incompatibility, it brings about the destruction of Rhesus-positive fetal erythrocytes and subsequent fetal anaemia and death if not treated (see p. 1407). In some mammals (oxen, sheep), antibodies are transferred to the offspring in the first formed milk (colostrum) after birth. In humans, maternal milk contains secretory immunoglobulins (IgA) which help to combat bacterial and viral organisms in the alimentary tract of the baby during the first few weeks, although it does not appear to be absorbed through the gut wall as it is in the mammals noted above.

T lymphocytes (9.12, 17, 18c)

T lymphocytes include a number of subclasses (see Marrack & Kappler 1986), all derived from stem lymphocytes originating in the bone marrow but later differentiating in the thymus (hence thymus-derived cell) and passing into various other lymphoid organs. They carry out a wide variety of *cell-mediated* defensive actions which are not directly dependent on antibody activity and are therefore included under the heading of *cellular immunity*. T-lymphocyte responses can be loosely divided into *effector* and *controlling* actions. *Effector responses* are direct and indirect attacks against virus-infected tissue cells, fungi, some protozoal infections (e.g. trypanosomes), neoplastic cells and the cells of grafts from other individuals (allografts) when the tissue antigens of the donor and recipient are not sufficiently similar. *Controlling functions* are the induction or suppression of immune responses in other lymphocytes, namely B cells and T cells engaged in effector responses, as well as a variety of non-lymphocytic cells such as those derived from the bone marrow. The effector T cells can be divided into two major classes, those which are responsible for cytotoxic killing of virus-infected cells, etc. and those which give rise to a combination of defensive actions through the release of certain soluble proteins, *cytokines*, in delayed-type hypersensitivity reactions.

Cytoxic T cells. These cause the death of their targeted cells in a number of different ways, including the release of toxic lysosomal proteins ('perforins') able to lyse the cell membranes of other cells by forming large pores. Such actions occur at close range, the effector cell having contacted the target cell and recognized it as being pathological. This recognition step is of great interest and depends on the presence at the surface of the target cell of a foreign antigen (e.g. part of a vital antigen) in combination with the Class I MHC molecules that are expressed on the cell surfaces of all nucleated cells. Class I MHC molecules are highly polymorphic and, although very variable from person to person, are invariant within all the tissues of an individual (p. 1407). It is thought that during ontogeny and selection of T lymphocytes in the thymus of an individual person, only those cells that are able to recognize the MHC I molecules characteristic of that individual are retained. Thus a part of the *T-cell antigen receptor* (also variable, see p. 1422) binds to MHC I molecules on other cells, recognizes them as self-components and leaves them unmolested. If, however, the other cells also present a foreign antigen (peptide component) in association with the MHC I molecule, or in the case of transplants, a protein expressed by a different MHC I allele, then T cells mount an attack on those cells which bear these foreign antigens. In this way, intracellular pathogens, such as viruses lodged within cells and thus beyond the reach of antibodies, and also perhaps 'transformed' neoplastic cells, can be killed. Of course, the same applies to the foreign cells of an allograft. Other 'lymphocytes' which are larger in size and contain cytoplasmic granules containing cytotoxic substances (*large granular lymphocytes*) can kill 'target' cells through similar mechanisms, but do not have T-cell receptors and do not act in an MHC-restricted fashion. Natural killer cells (see below) have a similar appearance to those described above.

Delayed type hypersensitivity-related T cells. These lymphocytes also react to the presence of antigens by synthesizing and releasing *cytokines*, soluble proteins with a molecular mass of 20–80 kDa, which have a wide variety of actions on other cells. These include *chemotactic substances* which attract macrophages into the area of release (macrophage chemotactic factor) and then prevent them from migrating away (macrophage inhibitory factor). Other cytokines stimulate phagocytosis and the destructive activities of macrophages (see p. 1420), natural killer and other cells (macrophage activating factors and γ-interferon). Another important cytokine is *interleukin 2* (IL-2), which acts on B cells and other T cells to stimulate their proliferation and maturation; there are also many other actions of this complex group of secretions. The phenotype of T cells responsible for this cell mediated type inflammatory reaction is predominantly CD4 positive, although some CD8 positive cells are commonly found on the periphery of the mononuclear cell infiltrate of cells in these lesions.

Helper T cells (CD4 phenotype). These are vital to cell proliferation and secretion of antibodies by mature B lymphocytes. These processes are initiated by a foreign antigen being phagocytosed and partially digested by an antigen presenting cell (APC) (p. 1415). The products of this antigen processing pass via the endosomal pathway of the cell to the APC surface where they are presented on Class II MHC molecules (a family of cell surface molecules found mainly on dendritic cells, macrophages, B lymphocytes and other antigen-presenting cells with similar functions) expressed on the cell

membrane. This combination of antigen and MHC II molecule is then presented at the cell surface to a helper T cell which recognizes the foreign peptide plus part of the Class II MHC molecule via its T-cell receptor. This interaction, together with secondary signals from cytokines released by the APC, and interactions with other cell adhesion molecules expressed on the two cells concerned, causes the activation and proliferation of the helper T cell. The T cell then activates B lymphocytes which are stimulated to differentiate into plasmacytes secreting antibody corresponding to the particular antigen involved in the APC–T-cell interaction. In this highly regulated way, clones of B cells that produce specific antibodies against an antigen can be stimulated to proliferate and secrete their products. Helper T cells are also required to supplement the activation of cytotoxic T cells, although a separate group of helper T cells is probably involved.

If such helper cell activities are destroyed or rendered non-functional (anergy), a state of immunodeficiency exists where potentially pathogenic organisms present in the body, but normally kept in check by the immune system, may proliferate, causing overt pathology and even death. A well-known example of this is the Acquired Immune Deficiency Syndrome (AIDS) where a virus (HIV I) specifically infects and kills predominantly helper T cells, but also a variety of antigen presenting cells. Similar secondary immunodeficiencies may result from other viral infections, from malnutrition, metabolic disorders, malignancies, drug therapies, radiotherapy and many other factors that suppress the cell-mediated immune system.

Suppression. There appear to be certain T cells which, when antigenically stimulated, release cytokines that actively suppress the defensive activities of B cells and of other T cells. The mechanism of their action is not well understood but may have features in common with both phenotypes of effector T cells (CD8 and CD4 phenotypes). The existence of both positive and negative controls in the immune system is of great significance, since for normal effective defence against infection, this system must be finely balanced to ensure destruction of foreign organisms without the body itself being damaged by the powerful agents released by the various defensive cells. Failure of this delicate control may be seen, at one extreme, in immunodeficiency states and, at the other, in responses to self-antigens such as in autoimmune diseases and in overactive responses called hypersensitivity reactions (e.g. allergic asthma, allergic dermatitis).

Structurally, T lymphocytes present different appearances depending on their type and state of activity. When 'resting' they are typical small lymphocytes morphologically indistinguishable from B lymphocytes; but when stimulated they become large (up to 15 μm), moderately basophilic cells with a partially euchromatic nucleus. In the cytoplasm are numerous free ribosomes, some agranular and granular endoplasmic reticula, a Golgi complex and a scattering of mitochondria (9.18c). Cytotoxic effector T cells also contain dense lysosome-like vacuoles used in cytotoxic killing (see above).

Natural killer cells. These constitute a pool of defensive elements which appear to have functional similarities to cytotoxic T cells, although they lack some typical lymphocyte features (see below). They normally form only a small percentage of all lymphocyte-like cells and are included technically in the 'large granular lymphocyte' category. Natural killer cells, when mature, have a mildly basophilic cytoplasm and a partially euchromatic nucleus. Ultrastructurally, the cytoplasm contains ribosomes, granular endoplasmic reticulum and dense, membrane-bound vesicles 200–500 nm in diameter with crystalline cores. These contain some hydrolases, but the major active component is a protein, cytolysin, capable of inserting holes in the plasma membranes of other cells, so causing their death. Natural killer cells are activated to attach themselves to and kill target cells of various kinds by a number of factors, including IL-2 from T cells (see above). They represent a relatively non-specific means of attacking virus-infected cells, protozoa and other pathogenic cells.

T-cell classes: CD nomenclature

When monoclonal antibodies were raised against cell surface antigens of human lymphocytes, it was found that different classes of T lymphocytes could be grouped together according to the characteristic range of monoclonal antibodies they bound. When these cell surface molecules were finally identified by the determinants recognized on them by monoclonal antibodies, they were given an International Cluster Determinant (CD) number. Nowadays, CD numbers define a very large number of cellular molecules which have been cloned and sequenced and whose biological functions are wholly or partially characterized (Singer et al 1994). All 'true' T cells express the CD3 molecular complex, which is responsible for signal transmission, mediated via the T-cell receptor (TCR) they also express either CD4 or CD8. Those which bear the CD4 molecule (CD4+) include helper-inducer cells important in triggering antibody production from B lymphocytes, cytotoxic T cells, and T cells involved in delayed hypersensitivity reactions. Those bearing the CD8 molecule (CD8+) comprise cytotoxic T cells and others with suppressor functions on other cell types. Natural killer cells are CD3 positive, but do not normally express CD4 or CD8 markers. This scheme of classification is by no means absolute in terms of relating T lymphocyte phenotypes to a particular function. At present the CD4 population has been subdivided into three subgroups (Th_0, Th_1, Th_2) based on the mix of cytokines released by these T cells following antigen stimulation. These CD molecular complexes are believed to act co-operatively with T-cell receptors, to mediate stimulus transduction and activation of a number of cellular functions. Both CD4 and CD8 molecules can be regarded as functioning as co-receptors to the TCR in the recognition of antigen, and are involved in the signal transduction from the cell surface to the nucleus to initiate 'helper' or other related activities, or in the case of CD8 to initiate cytotoxic activity, or 'suppressor' functions, etc. Although the plethora of activities carried out by lymphocytes seems highly complex, it is to be expected that the potent and wide-ranging defensive mechanisms of the body should be subject to multiple checks, controls and regulations. As yet, relatively little is understood about the manner in which the various parts of the whole system of cellular and chemical defences are **integrated**, but it is increasingly clear they must be viewed as a **single system** of great efficiency and elegance. When, however, such integration breaks down, the effects may be far reaching as, for example, in the wide variety of *autoimmune diseases* that occur in man, and in neoplasia of the immune system, such as myeloma.

Immunological memory

If after one antigenic response the body is again exposed to the same antigen, the second response is much more rapid and extensive, even after a period of years; this forms the basis of clinical immunization. This phenomenon implies the presence of some type of immunological 'memory'. It appears to be the result of a number of factors, which are at present not entirely clear, including the possible persistence of long-lived 'memory B and T cells'. However, there is growing evidence that it is the persistence of antigen in selected sites (e.g. follicular centres, associated by APCs) that is a major factor in immunological memory. Overreactivity of this system may be associated with various types of hypersensitivity, for example anaphylactic shock and delayed-type hypersensitivity.

The nature of antigenic response

The antibody–antigen reaction is **highly specific**, each antigen requiring its own antibody for binding to occur. In the life of an individual an enormous range of antigens impinges on the immunological system, requiring a correspondingly large number of antibody producing B lymphocytes and an equally large number of antigen-specific T lymphocytes to deal with them. Recent studies on the mechanisms of generating a wide variety of antibodies (antibody diversity) capable of recognizing any conceivable macromolecule which might challenge the body during infections, have indicated that control of this variability results from a number of different processes which operate during the formation and maturation of B lymphocytes. As already stated, antibody molecules consist of four polypeptide chain structures (two light and two heavy chains), and each of these has an antigen-binding end (Fab) of variable structure, as well as a constant (Fc) region. The constant and variable regions are coded for by separate genes, so that, for the IgG antibody, at least four genes are needed to code for a single molecule, two of which are variable and two constant. Different combinations from a limited number of variable region genes are, therefore, capable of producing a large number of different antibody specificities (antigen combining sites). In fact, there are families of variable (V) genes,

and each member of the family is constructed from many subunits which can be spliced together in different combinations to give a vast range of possibilities. To produce the heavy chain V region a functional V gene is compiled from three gene segments, V, D and J (Variable, Diversity and Junctional), and for the light chain V region there are V and J segments. Each V region gene is then linked to a constant region gene thus creating two genes to produce one polypeptide chain. The antibody diversity in the V region is further increased by somatic mutations in the genes, particularly in the heavy chain D regions during B-cell development, and probably by other factors such as variations in the transcription of DNA into messenger (m)RNA. It has been calculated that from as few as 200 V region genes (and segments) it is possible to generate at least 10^9 different antibody specificities by random combinations of these different components. Similar considerations apply to the T-cell receptor molecules.

The T-cell receptor

The T-cell receptor molecule consists of two polypeptide chains, either $\alpha\beta$ or $\gamma\delta$, which like immunoglobulin heavy and light chains have an N-terminal variable region and a C-terminal constant region encoded by a cluster of variable region gene segments and four constant region genes. In the bone marrow and later on in the thymus these genes are rearranged to generate the wide variety of T-cell receptors encountered. T-cell receptor responses to antigen are said to be MHC-restricted because they will only recognize an antigen when it is presented to them on an autologous (self) MHC molecule (either Class I or Class II) on other cells. However, during T-lymphocyte ontogeny the cell selects either an $\alpha\beta$ chain complex or a $\gamma\delta$ combination from the four germ line genes coding for the constant regions of these molecules.

Clonal selection theory

When the great diversity of antibody and T-cell specificities became apparent, the mechanism by which the body is able to produce the appropriate T- and B-cell responses to neutralize a particular antigen came under scrutiny. It has been shown that the lymphocyte populations of the body include B cells which already have the capacity to make almost any antibody and all types of T-cell specificity which might be required in the life of the individual. It has also been demonstrated that an antigen causes those cells which possess antigen receptors able to bind it, to each proliferate into a series of identical cells (a *clone*) that all produce the same antibodies and, in the case of T cells, that all have the same T-cell receptors. As an extension of this, it was postulated that clones inappropriate to defence, for example those able to produce antibodies against the body's own substances (auto-antigens) are in some way suppressed, being either eliminated by induced cell death (apoptosis) or kept quiescent (anergy). Of the huge range of possible antibodies that a B cell might synthesize, it is found in practice that only one is expressed in a particular B cell (*allelic exclusion*), and that its progeny will all express exclusively the same antibody specificity for antigen. When suitably stimulated, such B cells proliferate and mature into plasmacytes synthesizing identical antobodies. However, during secondary immune responses B cells usually switch the class (isotype) of the immunoglobulin from IgM to IgG or to some other isotype (e.g. IgA or IgE) under the direction of T-helper (CD4) control, but the specificity for antigen (defined by the V region of the molecule) remains the same. This 'class' switching enables antibodies to perform different biological functions (via their Fc regions) while retaining the same antigen specificity. Besides its importance in immunology, this phenomenon forms the basis for modern monoclonal antibody technology.

Specific T-cell responses to antigens are controlled in a similar way, in that T-helper and cytotoxic T cells, and perhaps other types of lymphocyte, are activated by interaction with antigen-presenting cells. The specificity to a particular antigen is determined by the T-cell receptor (TCR). Thus, like B cells, a great variety of T cells exists, each with a specific T-cell receptor preselected to recognize almost any antigen that might enter the body and enable the host to launch an immune attack upon it (either by itself, or by helper actions in co-operation with other defensive cells). When the antigen-MHC II complex at the surface of the antigen presenting cell interacts with the T-cell receptor complex, signals are transduced to the T-cell nucleus which lead to the proliferation of this clone and thus an amplification of the immune response. Cells not bearing the T-cell receptor corresponding to that antigen remain unresponsive. The existence of the same phenomenon in two cell types (B and T) is not accidental, since the control of most B-cell responses is regulated by helper T cells responding to the same antigen. B cells can also act as antigen presenting cells by taking in antigen via their surface antigen specific receptors and then processing it into small polypeptide pieces before presenting it on their cell surfaces in association with their Class II MHC molecules. T-cells then recognize this processed antigen via their specific T-cell receptors and respond by releasing an array of cytokines which act directly on the B cell, causing it to differentiate into a plasmacyte and secrete antibody.

Direct activation of B cells by antigen in the absence of T cells can also occur, but only by a narrow range of rather unusual antigens which can cross-link the B-cell receptors, for example by identical repeating epitopes on linear carbohydrate molecules such as pneumococcal polysaccharide, leading to the production of IgM rather than IgG antibody. This type of T-cell-independent response is of limited importance when compared to the main immune responses observed in vivo.

Immunological tolerance

Since lymphocytes react to foreign antigens and not usually to the proteins and carbohydrates of the body itself, there must be some mechanism which ensures the distinction of **self** from **non-self**, that is, an *immunological tolerance* to self. If a breakdown in self-tolerance occurs, the autoimmune sequelae may give rise to immunopathology such as autoimmune thyroiditis (Hashimoto's disease) where immune-mediated destruction of the thyroid gland occurs and the result is clinical hypothyroidism. Some evidence for such an immunological aetiology exists for a number of other chronic relapsing diseases, including disseminated sclerosis which causes demyelination of neurological tracts within the central nervous system. Self-tolerance is procured in man early on during fetal development, in the thymus and bone marrow (p. 1428) and continues throughout adult life in other peripheral lymphoid tissues and organs of the body in ways that are still not fully understood. Burnet widened the theory of selective antigenic response (see above) to attempt an explanation of the mechanism of self-tolerance (the *clonal theory*), suggesting that those lymphocytes capable of producing antibodies directed against the body's own tissues are suppressed in fetal life and are then no longer available to multiply at a later stage (see Burnet 1969; Edelman 1974). The reality, however, is that every individual has many B- and T-lymphocyte clones able to address a wide number of auto-antigens present in their immune repertoires, which in the majority of cases do not lead to overt disease and self-destruction. Therefore, there are several layers of carefully controlled regulatory mechanisms that keep in check immune responses directed against antigens, whether they be self-derived or from outside the body. These include:

(1) in the thymus (for T cells) and bone marrow (B cells), deletion of those lymphocytes that recognize 'self' peptides in association with 'self' MHC (see p. 1428);
(2) failure to provide the second signal necessary for lymphocyte stimulation following antigen recognition, for example lack of T-cell help for B cells, thus inducing a state of anergy on the 'self'-directed (autoreactive) cells;
(3) autoreactive cells that do not have the appropriate tissue-specific addressins to enable them to 'home' back to the organ or tissue which contains the relevant auto-antigen during lymphocyte recirculation (see p. 1423), and
(4) active suppression of autoreactive responses by T cells through their release of cytokines that down-regulate the expression of MHC molecules and receptors of other cytokines, for example IL-2 receptors.

Only when all these controls fail do auto-antigen-specific responses occur, and even then regulation can be partially restored, as seen in the chronic relapsing scenarios found in many of the so-called autoimmune diseases, for example rheumatoid arthritis, multiple sclerosis and autoimmune thyrotoxicosis (Grave's disease).

The principle of self-tolerance may also be put to clinical use when grafts of tissue between monozygotic twins or closely related

individuals are exchanged. All tissues from within a particular species have antigenic classes similar to those expressed in man as *histocompatibility* (HLA) tissue antigens. The success or failure of *isologous grafts* (i.e. grafts between genetically dissimilar members of the same species) depends largely on the degree of matching of these antigens between donor and recipient.

LYMPHOID TISSUES

Lymphocytes are concentrated in many sites in the body, typically at strategic sites which are liable to infection, or in possible routes for the spread of pathogens. The main areas of lymphocyte proliferation can be classed as either primary or secondary lymphoid tissue. To understand these terms it is necessary to examine how lymphocytes are formed and distributed in the body.

Circulation of lymphocytes (9.16)

The pluripotent stem cells for both B and T lymphocytes are present in the bone marrow where they proliferate to produce the precursors of both of these cell lines. For their differentiation and maturation, T-lymphocyte precursors have to pass into the thymus, which they do through the circulation; there, a number of major differentiation stages are passed through before the T cells are ready to engage in immune defence. From the thymus, the T cells enter the circulation again and pass to various peripheral sites where they can further multiply under the control of antigen-presenting cells. Likewise, B cells have to undergo a series of differentiation steps before they can perform their defensive roles. It appears that this generally occurs within the bone marrow. The newly formed B cells leave the bone marrow through the circulation and, like the T cells, pass to peripheral sites. The thymus and bone marrow are therefore described as *primary* (or *central*) *lymphoid organs* because of their initial roles in lymphocyte generation. The secondary or peripheral lymphoid organs or tissues include lymph nodes, spleen, lymphoid tissue associated with epithelial surfaces, such as the palatine and nasopharyngeal tonsils, Peyer's patches in the small intestine and various lymphoid nodules in the respiratory and urinogenital systems, the skin and conjunctiva of the eye. The bone marrow also serves as secondary lymphoid tissue as well as being primary, as lymphocytes pass back into it through the circulatory system, to engage in further proliferation when needed. In all the secondary tissue there are specific areas where either B or T cells are concentrated; cells enter them by migration through the walls of capillaries or venules and, having proliferated, leave by the lymphatic system or, in the case of the spleen, also by the venous drainage. The lymphocytes are now ready for action, and may be distributed to many other sites in the body when the need arises.

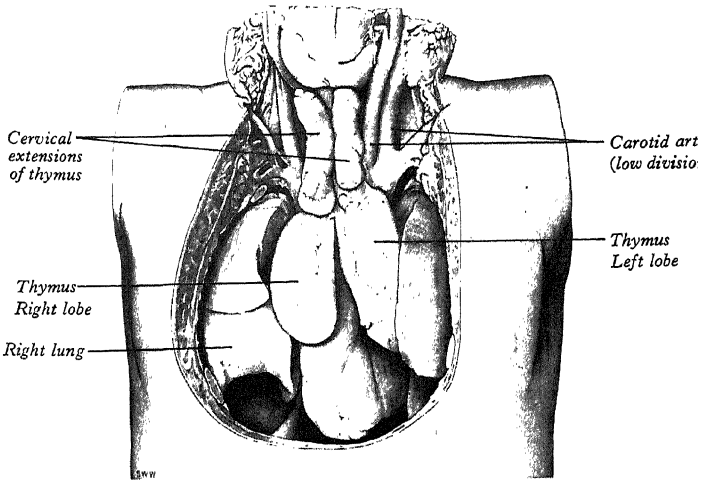
In the present account the thymus will be described first, followed by examples of the secondary lymphoid tissues and organs and then the spleen. The bone marrow has already been described earlier in this section (p. 1409).

THYMUS (9.19–28)

The thymus (**9.19**) is one of the two primary lymphoid organs (the other being the bone marrow). It is responsible for the provision of thymus-processed lymphocytes (T lymphocytes) to the whole body. The thymus provides a unique microenvironment in which the T-cell precursors (thymocytes) undergo development, differentiation and clonal expansion; during this process, the exquisite specificity of T-cell responses is acquired, as also is their immune tolerance to the body's own components. These steps involve intimate interactions between thymocytes and other cells (mainly epithelial cells and antigen-presenting cells) and chemical factors of the thymic environment. The organ is also part of the neuroendocrine axis of the body, and it both influences and is influenced by the products of this axis. Its activity, therefore, varies throughout life under the influence of different physiological states, disease conditions and chemical insults such as drugs and pollutants.

THYMIC ANATOMY

The appearance of the thymus varies considerably with age. It is largest in the early part of life (**9.19**) up to the age of about 15, although it persists actively into old age (see p. 1429). It is a soft, bilobed organ, its two parts lying close together side by side, joined in the midline by connective tissue which merges with the capsule of each lobe. In children it is more pyramidal in shape and firmer than in later life, when its lymphoid content is reduced. In the fresh state (**9.20**) it is deep red due to its rich vascular supply; with age it becomes thinner and greyer before yellowing as adipose tissue infiltrates the organ, a process which is independent of obesity. Its weight also varies with age (see p. 1429); at birth it is 10–15 g and rapidly increases to about 20 g, then remains at that level thereafter, although the amount of lymphoid tissue gradually decreases (see **9.30**, and below). Each of the two lobes is partially divided by the ingrowth of shallow septa so that superficially it appears lobulated; as fatty atrophy proceeds during ageing this lobulation becomes more distinct. The older thymus can be distinguished from the surrounding mediastinal fat only by the presence of its capsule, although even within greatly atrophied glands there are usually greyer areas around blood vessels, formed by persistent lymphoid tissue.



9.19 Dissection to display the neonatal thymus.

Position and relations

The greater part of the thymus lies in the superior and anterior inferior mediastinum, the lower border of the thymus reaching the level of the 4th costal cartilages. Superiorly, extensions into the neck

9.20 Human thymus from a 9-year-old girl (left) and an 80-year-old man (right). Note the fatty infiltration of the older thymus. (Provided by M Kendall, Department of Pharmacology, UMDS, St Thomas's Campus, London. Reproduced with permission from Kendall 1981.)

are common (9.19), reflecting the (bilateral) embryonic origins of the thymus from the third pharyngeal pouch (see p. 1428); it sometimes reaches the inferior poles of the thyroid gland or even higher. Its shape is largely moulded by the adjacent structures. **Anterior** are the sternum, adjacent parts of the upper four costal cartilages and the sternohyoid and sternothyroid muscles. **Posterior** are the pericardium and the aortic arch with its branches, the left brachiocephalic vein and the front and sides of the trachea. Ectopic thymic tissue is found in 25% of the population (Goldstein & MacKay 1969); small accessory nodules may occur in the neck representing portions which have become detached during their early descent, or the thymus may be found even more superiorly as thin strands along this path, reaching the thyroid cartilage or above. Connective tissue marking the line of descent during early development may, in some instances, run between the thymus and the parathyroids.

Vessels

Arteries. These are derived mainly from internal thoracic and inferior thyroid artery branches which also supply the surrounding mediastinal connective tissue, although a branch from the superior thyroid artery is also sometimes present. There is no main hilum but arterial branches pass either directly through the capsule or, more often, into the depths of the interlobar septa before entering the thymus at the junction of the cortex and medulla.

Veins. These drain to the left brachiocephalic, internal thoracic and inferior thyroid veins; one or more veins often emerge medially from each lobe of the thymus to form a common trunk opening into the left brachiocephalic vein.

Lymphatics. Afferent lymphatics are absent from the thymus but efferent lymphatics arising from the medulla and corticomedullary junction drain through the extravascular spaces in company with the arteries and veins entering and leaving the thymus. In rodents, large lymphatic vessels draining to perithymic lymph nodes are often found within the subcapsular cortex but these also receive lymph from other areas of the body; these lymph nodes drain in turn to neighbouring regional nodes (Goldstein & MacKay 1969). Whether there is a similar perithymic lymphatic drainage in humans remains unknown.

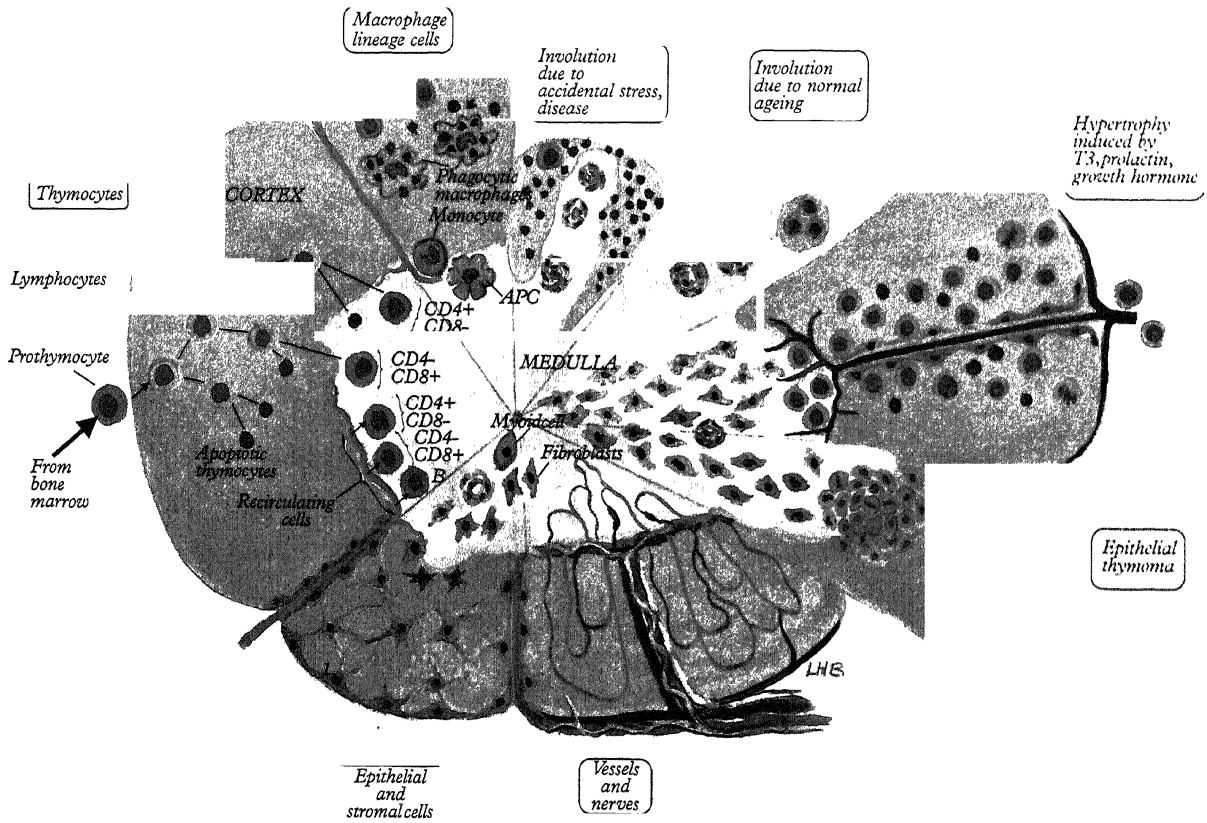
Innervation

Thymic innervation is derived from the sympathetic chain via the cervicothoracic (stellate) ganglion (or from the ansa subclavia) and the vagus. Branches from the phrenic nerve and descendens cervicalis are distributed mainly to the capsule of the thymus. During development (Hammar 1935), vagal innervation of the thymus commences in the neck before its descent into the thorax. The two lobes are innervated separately through their dorsal, lateral and medial aspects and rich neural plexuses are formed in the medulla. After its descent, the thymus receives the sympathetic nerves along vascular routes, their terminals branching radially and forming with the vagal fibres a plexus at the corticomedullary junction. Innervation is complete by the onset of thymic function. While many of the autonomic nerves are doubtless vasomotor, many terminal branches also (at least in rodents) leave their perivascular pathways and pass among the cells of the thymus, particularly the medulla, suggesting that they may have other roles. The medulla also contains a variety of non-lymphoid cells, including cells positive for vasoactive intestinal polypeptide (VIP), acetylcholinesterase (AChE) large, non-myoid cells and cells containing oxytocin, vasopressin and neurophysin, with possible neural crest origin. Clearly, the roles of the nervous system and other neuroendocrine elements in the overall biology of the thymus are far from being understood and suggest many intriguing possibilities.

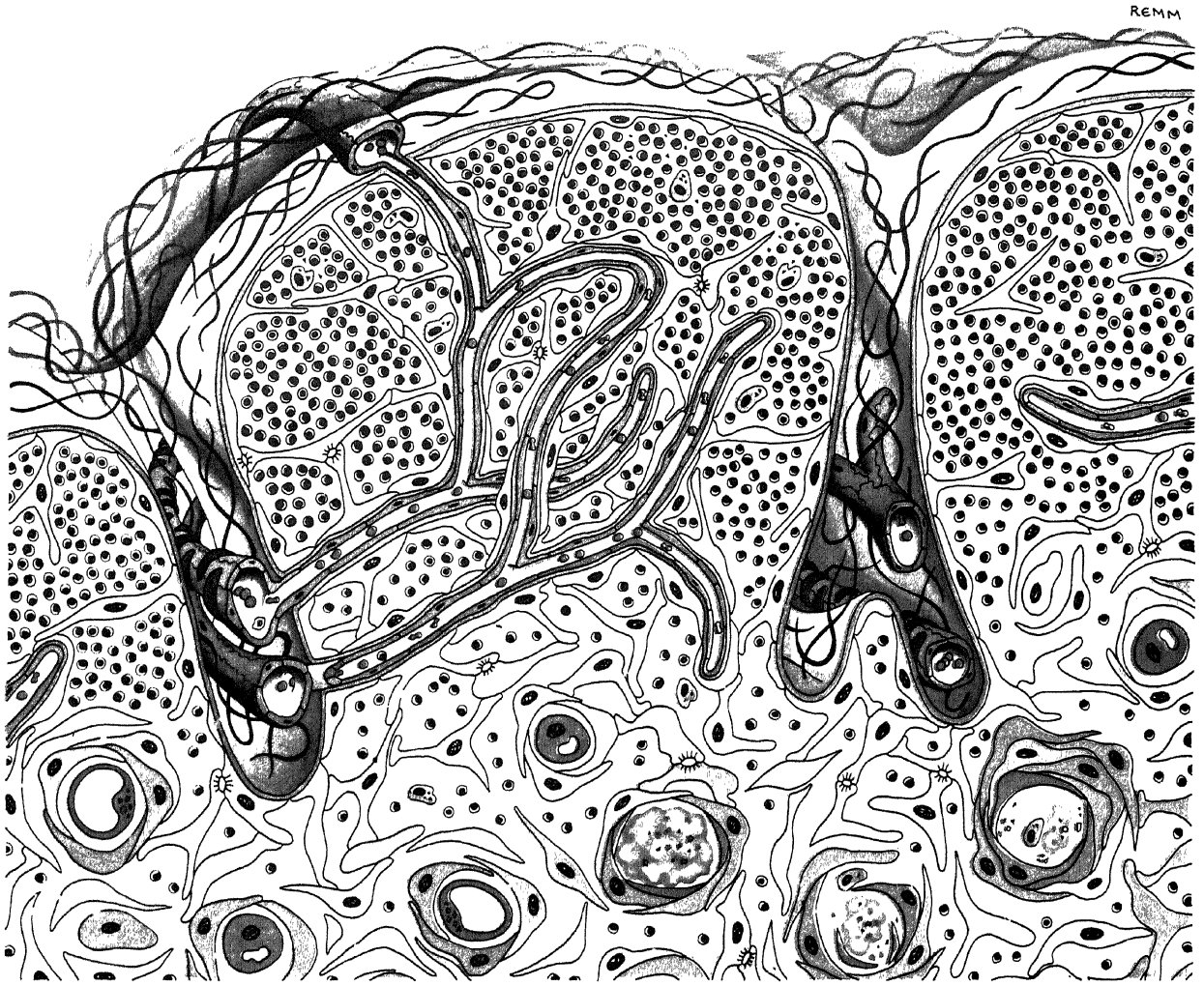
THYMIC MICROSTRUCTURE (9.21–31)

General architecture

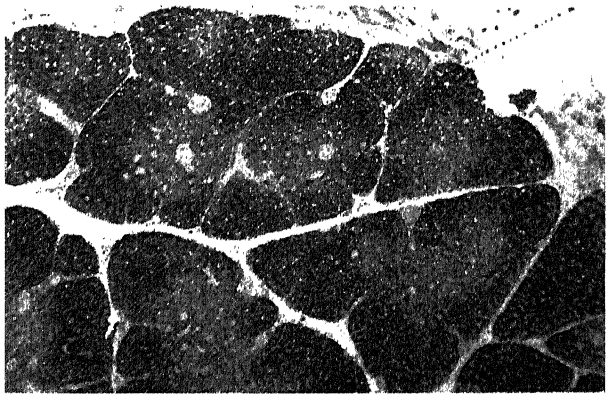
To understand the cellular organization of the thymus it is helpful to consider its embryonic origins. The thymus is derived from a



9.21 Schema illustrating the microscopic organization of the thymus at various stages of life and under different conditions.



9.22 A scheme of thymic structure; the various elements are not drawn to scale to enable representation in a single diagram. Note the lobular outlines, capsule, delicate interlobular septa, cortical lymphocytes, the epithelial 'reticular' cells and their junctions and the medullary corpuscles of Hassall showing a graded series of increasing maturity; also the transcortical circulation. See text for further discussion.



9.23 Survey photograph of a neonatal human thymus stained with haematoxylin and eosin. The general lobular architecture is seen; each lobule contains a relatively pale medullary core surrounded by a densely cellular, dark, heavily stained cortex. (From a specimen prepared and provided by R O Weller, Department of Pathology, Guy's Hospital Medical School.)

number of sources, including epithelial derivatives of the pharyngeal wall, mesenchyme, haemolymphoid cells and vascular tissue. These form distinctive components within the mature thymus, interacting functionally to create its unique immunological properties.

When sectioned (**9.20–24**), the thymus is seen to consist of an outer *cortex* of densely packed cells mainly of the T-lymphocyte lineage, the *thymocytes*, and an inner *medulla* rich in connective tissue but with fewer lymphoid cells. Both lobes have a loose fibrous connective tissue capsule, from which septa penetrate to the junction of the cortex and medulla, to partially separate the irregular lobules, each 0.5–2.0 μm in diameter. A loose network of interconnected *thymic epithelial cells* permeates the cortex and medulla. In each lobule, the cortex has a superficial *outer cortical region* (subcapsular cortex) composed of a narrow band of cells immediately beneath the capsule, and the main cortex which is much more extensive. The central core of both thymic lobes is composed of a *medulla* which is continuous from one lobule to the next. The lobulations are partially separated from each other by connective tissue septa that form a route of entry and exit for blood vessels, efferent lymphatics and nerves. Most cells enter or leave the thymus by this route.

Epithelial framework (9.21–23, 25–27)

Unlike other lymphoid structures, where the supportive framework is chiefly collagenous reticular tissue, the thymus is permeated by a network of interconnected epithelial cells (*thymic epitheliocytes*) between which lodge lymphoid and other cells of the organ. There

is only a little reticulin and few fibroblasts. By cell–cell contact and the release of soluble factors, the epitheliocytes create the microenvironments necessary for the thymocytes to develop.

Epitheliocytes vary in size and shape in the different positions within the thymus. Typically they have pale, oval nuclei, a rather eosinophilic cytoplasm and desmosomal attachments between cells. Intermediate (keratin) filament bundles lie within the cytoplasm. These cells form a continuous external lining to the thymus beneath its fibrous capsule, following its lobulated profile and investing the vessels which pass into it. Other cortical epitheliocytes are branched, with large spaces between them, while those of the medulla tend to form more solid cords as well as the characteristic whorls of (often) partially keratinized stratified epithelium (thymic or Hassall's corpuscles). Lymphocytes lie within the meshes and cords formed by these various cells. There is much evidence that many distinctive functional roles are subserved by the epithelial cells (Ritter et al 1985), some of them related to the differentiation of T lymphocytes, others to the production of soluble thymic factors or hormones or to barrier and mechanically supportive functions (see Wijngaert et al 1984). Of special significance, however, is their role in MHC restriction of T-cell immune responses.

In the human thymus, the epitheliocytes have been divided morphologically into Types 1–6 (Wijngaert et al 1984) and also characterized immunohistochemically (**9.27**A, B; Lampert & Ritter 1988). There is considerable heterogeneity within these classes, and the two methods of analysis give slightly different results. Immunological reagents generally distinguish subcapsular, cortical and medullary epitheliocytes as well as Hassall's corpuscles. Some subcapsular and medullary cells share the same epitopes. According to the morphological classification (**9.25**), Type 1 epitheliocytes (subcapsular-perivascular) create the continuous monolayer around the perimeter of the thymus, extending along the septa to the corticomedullary boundary and forming an outer limit to the perivascular spaces. Capillaries within the cortex are similarly ensheathed, but medullary blood vessels are not (Kendall 1989). Type 1 cells are flattened, have a distinct basal lamina and are connected to adjacent cells by desmosomes. Morphologically they are distinguished from Type 2 cells by their shape and the lower content of short lengths of granular endoplasmic reticulum, fewer small electron-dense granules and the presence of a distinctive tubular complex of unknown significance. Like most other thymic epitheliocytes, Type 1 cells have MCCII-positive surfaces, apart from their capsule-facing aspects which are MHCII-negative. Type 1 cells secrete factors (e.g. β2-microglobulin) that attract stem cells to the thymus (Dargemont et al 1989), and thymic hormones (Dardenne & Savino 1990).

Type 2 cells extend from the outer cortex towards the medulla forming a series of cells in contact with Types 3 and 4 epithelial cells. Types 2 and 3 cells are large, with long cytoplasmic extensions (sometimes extending 100 μm from the nucleus). They are active cells with numerous cytoplasmic vesicles, several small Golgi bodies and many small electron-dense granules. Type 2 cells are more electron lucent than Type 3 cells which are, in turn, paler than the smaller very dense Type 4 cells. All cortical epitheliocytes are closely apposed to thymocytes, sometimes apparently engulfing them (emperiopoleisis). Large epitheliocytes with many associated thymocytes (50 or more) are called *thymic nurse cells* (TNC). These may be a special subset of Type 2 or 3 cells. They also contain mRNA for oxytocin and vasopressin, unlike most other cortical epithelial cells.

Type 5 cells are a small subset of medullary epithelial cells that appear to be relatively unspecialized. Type 6 cells are the commonest in the medulla, although several subsets may occur. Their forms range from spindle-shaped cells secreting thymic hormones to flattened cells forming Hassall's corpuscles.

Hassall's corpuscles are balls of flattened medullary epithelial cells from 30 to 100 μm in diameter which are characteristic features of the thymus (**9.25, 26, 29**). They start to form before birth and their numbers increase throughout life, often showing periods of increase or decrease. Their function is not clear, although in the past it has been suggested that they are graveyards for thymic cells (Blau 1969), or regions where immunoglobulins are concentrated. The centre of the corpuscle often contains cellular debris and sometimes eosinophils. Corpuscles with a similar appearance have been described in the palatine tonsil (p. 1444).

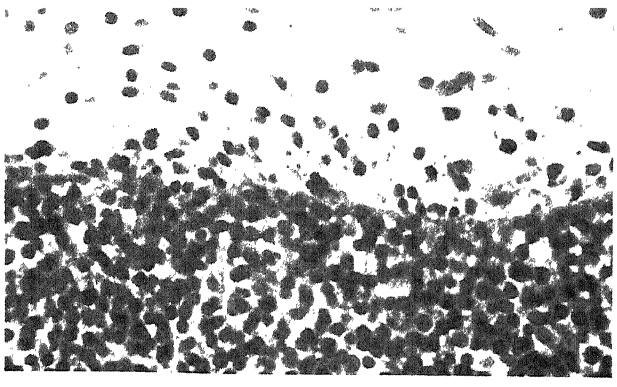
Some thymic epitheliocytes can be immunostained with antibodies against neuropeptides (Batanero et al 1992) and in rodents, cells with phenotypic and biochemical markers for both neurons and epithelial cells have been described in thymic cultures.

Other non-lymphocytic thymic cells

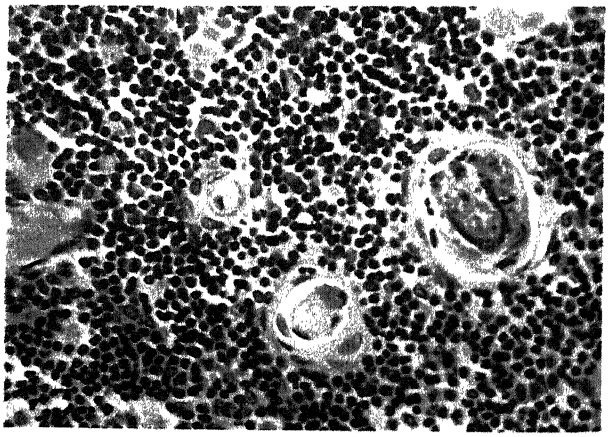
These include cells of the mononuclear phagocyte system, fibroblasts and myoid cells.

Cells of the mononuclear phagocyte system (macrophage lineage cells). These are found as *monocytes* at the corticomedullary junction, as *mature macrophages* in the cortex and as *interdigitating cells* in the medulla. In rodents, two types of cortical macrophages have been described, one a phagocytic cell capable of engulfing dying (apoptotic) thymocytes, the other producing proliferative factors for thymocyte development. The interdigitating cells are antigen-presenting cells similar to those found in other lymphoid organs, and are thought to be able to present antigens to the maturing T cells as they migrate from the cortex into the medulla, the medulla acting as a secondary lymphoid organ in this respect. Interdigitating cells are large, with characteristic infoldings of the plasma membrane, and do not generally contain phagocytic inclusions.

Fibroblasts. They are found in the capsule, perivascular spaces and medulla, but are infrequent in the cortex, except in involuted glands. Short-range or contact interactions between thymocytes, Class II MHC positive epitheliocytes and mesenchymal cells (or a



9.24 Prothymocytes entering the thymus rudiment through the capsule before blood vessels and nerves have invaded the epithelial cells. Haematoxylin and eosin. Magnification x 240. (Provided by M Kendall, Department of Pharmacology, UMDS, St Thomas's Campus, London.)



9.25 Neonatal thymus. A medullary field showing three concentric corpuscles of Hassall of varying degrees of maturity, surrounded by many closely packed lymphocytes and a number of reticular cells.

fibroblast cell line) have been found to be necessary for the in vitro development of thymocytes (Anderson et al 1993).

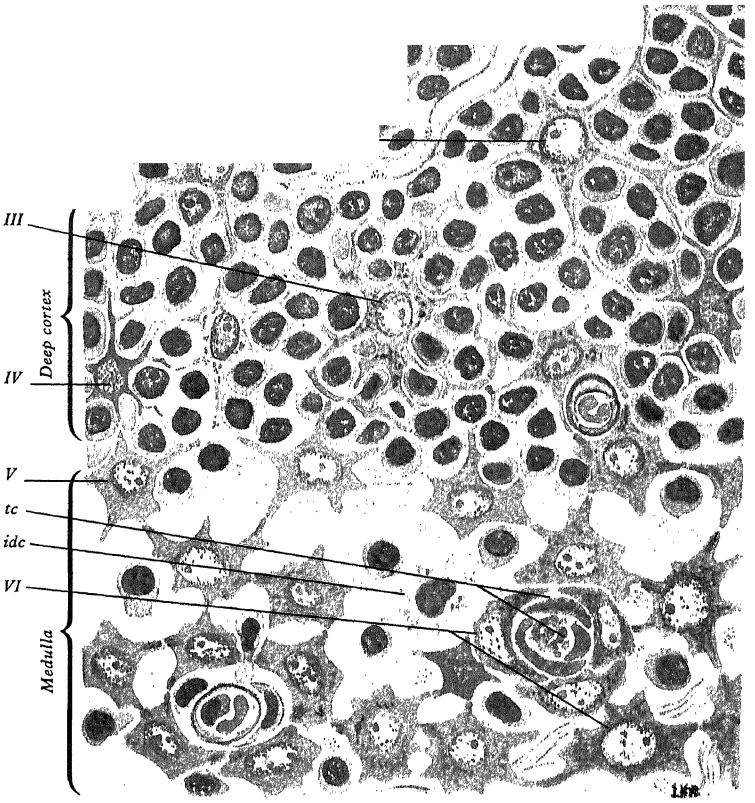
Myoid cells. These cells are situated mainly in the medulla and at the corticomedullar junction. They are large, rounded cells, with a central nucleus surrounded by irregularly arranged bundles of myofilaments. In lower vertebrates, where myoid cells are often more numerous, these cells are joined to neighbouring medullary epithelial cells by desmosomes. Their functions are unknown, although it has been suggested that their contractions might aid the movement of lymphoid cells across or out of the gland.

Lymphocytic population, thymocytes (9.21, 24–28)

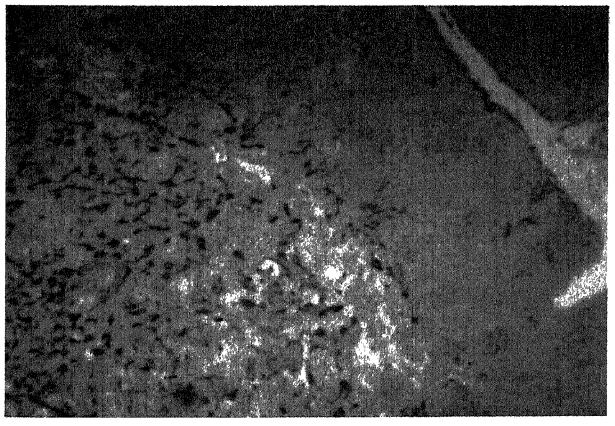
In the cortex, massive numbers of densely packed small thymocytes (thymic lymphocytes) predominate, occupying the interstices of the epithelial reticulum, which in histological sections they largely obscure, and forming about 90% of the total weight of the thymus. A distinct subcapsular zone is present, housing the thymic stem cells, prothymocytes and lymphoblasts undergoing mitotic division. The first stem cells to enter the thymus in the embryo come from the yolk sac and liver during their haemopoietic phases, possibly, as in birds, being attracted by thymic chemotactic substances. During later periods it is probable that all thymic lymphocytes originate in the bone marrow, or at least have sojourned there, before passing in the bloodstream to the thymus.

The cortex has two rather ill-defined zones: an outer cortex with a framework of Types 1–3 epitheliocytes and a deep cortex where Type 4 cells occur. Thymocytes undergo mitosis in all cortical zones as the clones of differentiating T cells mature, gradually moving deeper in the cortex. In rodents, cell cycling times of 8 hours have been recorded in the outer cortex, but no estimates exist for the human thymus. The appropriate conditions for the proliferation and differentiation of thymocytes appear to be produced by their close proximity to neighbouring epitheliocytes (see Janossy et al 1986). Although the nature of these interactions is not clear, it may involve the release from the epitheliocytes of soluble mitogenic and differentiation factors as well as induction of changes through intercellular contact. During this process, thymocytes differentiate along the T-cell line, acquiring the CD3+ marker and T-cell receptors, and also switching into different subclasses of T cells (p. 1420). The great range of different T-cell receptor types, running into many millions, is also established here by the expression of variable genes and related mechanisms (p. 1420).

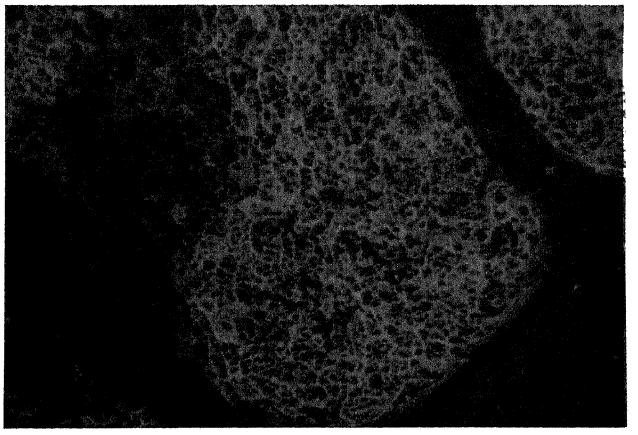
As time passes, the differentiating thymocytes enter the medulla, and migrate through the walls of venules and lymphatics to move into the circulation. Such cells (post-thymic thymocytes) are not immunocompetent within the cortex, and in general attain maturity only after entering the medulla or perhaps not until they reach their secondary lymphoid tissue destinations. However, the existence of antigen presenting cells and plasma cells in the medulla indicates



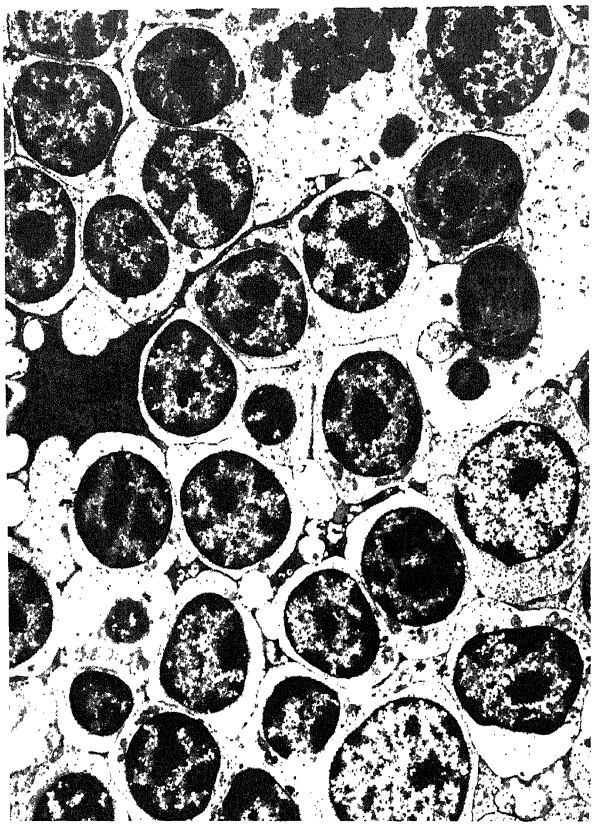
9.26 Cellular organization of the thymus showing thymocytes (blue) and epitheliocyte framework (green) of Types 1–6 cells. nf = nerve fibre; tc = thymic corpuscle; idc = interdigitating cell; bv = blood vessel.



9.27A Human pediatric thymus immunostained to show the distribution of the IL-4 receptor on Types 2 and 3 epithelial cells of the cortex. Peroxidase method. (Provided by Mary Ritter, Royal Postgraduate Medical School, London.)



9.27B Human pediatric thymus, near serial section corresponding approximately to 9.27A, immunostained to show thymulin-containing cells beneath the capsule (Type 1 epitheliocyte) and in the medulla (Type 6 epitheliocyte). Immunofluorescence (FITC) method. (Provided by Mary Ritter, Royal Postgraduate Medical School, London.)



9.28 Transmission electron micrograph of the thymic cortex showing lymphocytes ensheathed in epitheliocytes. Magnification × 3000. (Provided by M D Kendall, UMDS, St Thomas's Campus, London.)

that T lymphocytes **can** be activated within the thymus, if not in large numbers.

Thymocyte classes. Four major lymphoid cell types are found in the thymic cortex, as determined by immunocytochemistry and flow cytometry; each of them has a different proportion of small, medium and large thymocytes. Firstly, there are cells which do not express the mature T-lymphocyte markers CD4 or CD8 (double negative cells) nor, initially, CD3. Most of these are large *blast cells*, which after undergoing mitosis become small, double negative thymocytes and begin to develop the T-cell receptor (TCR) complex and become CD3-positive. These blast cells are primarily located in the subcapsular cortex and around blood vessels, especially at the corticomedullary junction. During the development of the TCR the cells give a transient expression of γ/δ, followed by α/β and other TCR components (see p. 1421), and simultaneously become double positive for CD4 and CD8, as well as the histochemical marker TdT (terminal deoxynucleotidyl transferase), characteristic of thymocytes thereafter (see Janossy & Campana 1989). The majority of these double-positive cells are small cortical thymocytes comprising 80–90% of the total thymocyte population. It is thought that the functional abilities of the T-cell repertoire is determined at this stage, with 'undesirable' lymphocytes dying in great numbers by apoptosis. The few that are rescued by the action of factors from the microenvironment (positive selection) become either CD4- or CD8-positive (i.e. single rather than double positives); these cells are found in the medulla and are slightly larger than cortical thymocytes.

Medullary single-positive thymocytes may either be cells about to be exported to the periphery where they will become fully immunocompetent, or recirculating activated T cells which have entered the medulla secondarily (Agus et al 1991). In addition, a few single positive cells represent early cortical cells that transiently express either CD4 or CD8 before becoming double-positive early thymocytes, as noted above.

In addition to these T-cell products, the thymus is also thought to be responsible for generating natural killer cell lineages.

The thymus often contains some immature B lymphocytes in the medulla or around blood vessels at the corticomedullary junction, and mature B cells (plasmacytes) throughout the thymus and in perivascular spaces. These cells are not formed in the thymus but arrive by immigration through the vasculature. The occurrence of occasional mature B lymphocytes and also, sometimes, germinal centres in the medulla also supports the conclusion that at least part of the medulla is more like a secondary ('peripheral') as opposed to a primary ('central') lymphoid tissue, receiving previously differentiated lymphocytes capable of immune interactions.

Acquisition of self-tolerance

As indicated above, the great majority of thymocytes never leave the thymus. However, during their maturation, thymocytes acquire the ability to recognize the HLA (MHC) markers expressed by the unique genome of the individual they belong to, i.e. 'self-antigens', so that when they migrate to the peripheral lymphoid organs and other tissue sites, they and the clone of cells they give rise to can detect alien antigens when these are present in association with 'self'-MHC determinants (see p. 1407) and can mount an appropriate immune attack. It appears that the thymocyte's correctness of response to self-MHC antigens is in some way determined in the thymic cortex before release and that if they respond inappropriately, the thymocytes are deprived of appropriate growth factors and undergo apoptotic death. In this way the thymus selects only T cells which can recognize alien antigens in combination with self-MHC molecules (i.e. they become MHC-restricted, see p. 1407).

Embryonic origins of the thymus

Most embryological evidence at present favours the view that the thymic epithelium is derived from both the ectoderm and the endoderm of the third and often the fourth branchial clefts and pharyngeal pouches; these layers interact with the associated neural crest mesenchyme at about the 10 somite stage (day 23; see Hammar 1911; Weller 1933; Norris 1937; Hamilton et al 1972; Stark 1975) to initiate thymic development.

The first stages of thymic development are seen bilaterally in the third pharyngeal pouch towards the end of the 6th gestational week, when endodermal cells form a sacculation. As these cells move caudally and anterolaterally they become surrounded by mesenchymal and ectodermal cells (Norris 1938), attracting prothymocytes which enter through the surrounding mesenchymal capsule. By the 8th week the two advancing thymuses are united in the midline, and basophilic thymic stem cells and thymocytes come to lie between the epitheliocytes (von Gaudecker 1986), which are visibly differentiated. Myoid cells appear in the centre of the thymus.

With the descent of the thymus, vascularization begins, and nerve fibres from the vagus grow in along the blood vessels, following the inward growth of connective tissue septa which produce a lobulated form. By 10 weeks, over 95% of the cells belong to the T-cell lineage, with a few developing erythroblasts and B lymphocytes. Hassall's corpuscles are also present. By 12 weeks, the mesenchymal septa, blood vessels and nerves have reached the newly differentiating medulla, allowing the entry of macrophage lineage precursors, macrophages and interdigitating cells being first seen at 14 weeks. Granulopoiesis occurs in the perivascular spaces. The 17-week thymus appears fully differentiated, and after this time it produces the main type of thymocyte also present throughout life (designated TdT^+).

In the mutant mouse *nude*, where the ectodermal anlage for the thymus is deficient, the thymus is abnormal, cystic and does not support lymphopoiesis, although lymphocytes are still produced in the bone marrow (Pritchard & Micklem 1973; see also Cordier and Haumont 1980). Developmental abnormalities of neural crest derivative that also affect the development of the heart and peripheral neural ganglia also result in thymic deficiency, as seen in the Di George and Pierre Robin syndromes (Couly et al 1983).

Microcirculation and local innervation (9.21)

Vessels in the cortex. The pattern of blood flow differs in the cortex and medulla. Major blood vessels enter the gland at the corticomedullary junction and pass within each lobe giving off small

capillaries to the cortex and larger vessels to the medulla. Most cortical capillaries loop around at different depths in the cortex and join venous vessels at the corticomedullary junction, but some continue through the cortex to exit from the thymus through the capsule and join larger veins running in the capsule. These smaller capillaries usually have a narrow perivascular space which sometimes contains pericytes and other cells, but rarely nerves.

There is a complete sheath of epithelial cells between the perivascular spaces and the cortex, an arrangement which has been called the blood-thymic barrier (Marshall & White 1961; Raviola & Karnovsky 1972). In the past it was assumed that thymocytes needed to be 'educated' in an antigenically pure environment. This was thought to be achieved in the cortex, since antigens in the blood would have to cross blood-vessel endothelium, the connective tissue of the perivascular space and an epithelial sheath in order to reach the lymphoid tissue of the cortex. More recently it has been shown that antigens can reach the cortex by a transcapsular route (Nieuwenhuis 1990). In the light of this finding it can be postulated that developing thymocytes are bathed in numerous antigens which could influence thymocyte education and subset formation (see below).

Vessels in the medulla. Medullary blood vessels are not so protected by epithelial cells, and those of the corticomedullary junction are only partially ensheathed (usually on their cortical aspect). Medullary vessels are surrounded by connective tissue and at certain times, for example in mid to late pregnancy (in rodents) the medulla may contain increased numbers of fibroblasts and connective tissue matrix. Medullary vessels are very variable in size, and some may have short lengths of high endothelium similar to those in lymph nodes and mucosa-associated lymphoid tissue.

Lymphatics. Efferent (though not afferent) lymphatics are also found in the perivascular spaces; they appear to begin in the medulla or at the corticomedullary junction.

Innervation. Most thymic nerves are seen near blood vessels, either in close apposition to them or forming complex ramifications in the tunica adventitia, or free in the perivascular spaces. Few nerves have been seen within the cortex or medulla in man. In rodents, however, subcapsular and corticomedullary nerve plexuses are present, and branches have been seen peeling off to enter the cortex to ramify between epithelial cells, macrophages and thymocytes (Felten & Felten 1989; Weihe et al 1989; Kendall & Al-Shawaf 1991). This has been disputed by other researchers (Novotny et al 1990).

Many nerve fibres in the major nerve bundles and plexuses are noradrenergic and therefore efferent sympathetic. Some nerves give a positive reaction with antibodies to vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), substance P (SP) or calcitonin gene-related peptide (CGRP), and are presumably sensory or sensorimotor. Occasionally large neuronlike cells with either CGRP positively or acetylcholinesterase (AchE) activity have been reported in the medulla. The presence of cholinergic nerves is in dispute (Bulloch & Moore 1981; Nance et al 1987; Tollefson & Bulloch 1990), and therefore the vagal fibres may be afferents.

Thymic haemopoiesis

Haemopoietic cells are present in fetal life, when the thymus makes an important contribution to the formation of erythrocytes and leucocytes. Later haemopoietic cells are often present, possibly as a result of the reactivation of persistent fetal stem cells. Normoblasts have been found in the thymuses of many adult open-heart surgery patients, and immature eosinophils, neutrophils and mast cells have also been observed. Where present in adults, the erythropoietic foci are mainly in the subcapsular and outer cortex.

Thymic changes during postnatal life (9.21, 30)

The thymus is largest relative to body weight at birth. It was previously believed that it increased in size until puberty after which it declined dramatically (Bratton 1925; Young & Turnbull 1931; Boyd 1932). However, many of the studies giving rise to this conclusion were based on post-mortem findings after illnesses of varying durations, and several authors comment that the thymus weights recorded were, therefore, probably underestimates. Studies of thymus weight after sudden death (Kendall et al 1980; Steinmann 1986) have recorded a wide variation at all ages, but the general pattern is that after the first year of life, when there is an increase, the mean weight is fairly constant at about 20 g until the 6th decade when a reduction occurs. Computed tomography and imaging studies of the thorax have given similar results.

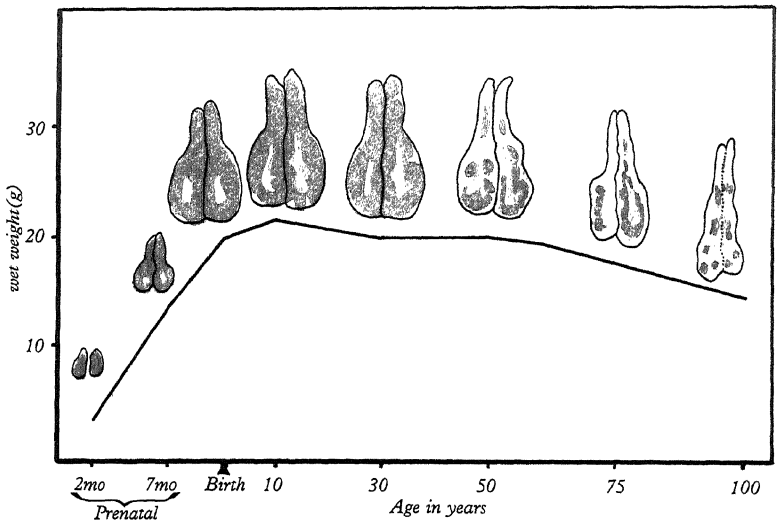
Although the weight of the thymus may be fairly constant, it is increasingly infiltrated by adipose tissue and so the total amount of active lymphoid tissue becomes progressively smaller. At birth, individual adipocytes may be seen in connective tissue septa, and increased numbers are found within the cortex in the second and third decades. Fatty infiltration is usually complete by the fourth decade when only the medulla and small patches of associated cortex are spared. Thymic hormone-secreting cells in the medulla persist throughout life.

Because of these changes, the numbers of thymocytes present must be greatly reduced in old age, as has also been found in cultured tissue. However, thymocyte production and differentiation persist throughout life, and T cells from this source continue to populate the peripheral lymphoid tissue, blood and lymph (see Steinmann & Müller-Hermelink 1984b).

THYMIC HORMONES AND OTHER SECRETED FACTORS (9.31)

The well-characterized thymic hormones are principally thymulin, the thymosins, thymopentin and thymic humoral factor. Thymulin, originally called 'Facteur Thymique Sérique' (FTS) relies on zinc for its biological activity. The thymosins are a large family isolated from thymosin fraction 5 (Goldstein et al 1977). The precursor of thymosin $\alpha 1$, prothymosin, is found in highest quantities in the thymus, but is also secreted elsewhere (Haritos et al 1984) as is parathymosin.

9.29 Electron micrograph of a section through thymic medullary epithelliocytes forming a Hassall's corpuscle. Rat. (Micrograph provided by M Kendall, Department of Pharmacology, UMDS, St Thomas's Campus, London.)



9.30 Diagram illustrating the age-related changes in thymic weight and composition. Pink = lymphoid medulla; red = the cortex, yellow = fatty infiltration. (After Kendall et al 1981 with permission.)

Thymosin $\beta 1$ is ubiquitin (Schlesinger et al 1975), and thymosins $\beta 4$ and $\beta 10$ have recently been shown to be sequestering components of connective tissue (Yu et al 1993). Thymopoietin, although originally studied for its neuromuscular effects, has its biological and immunological activity in residues 32–36 (thymopentin or TP5) (Goldstein et al 1979). Thymic humoral factor (THF) had also been sequenced (Burstein et al 1988) and no homology has been found between any of the thymic hormones.

The peptide hormones of the thymus have a range of immunomodulatory effects on lymphocyte maturation within the thymus and in the periphery (reviewed in Trainin 1974; Dardenne & Bach 1988; Safieh-Garabedian et al 1992). Most will induce markers of early differentiation on lymphoid cells lacking such markers, and enhance various T-cell functions. The injection of most thymic hormones restores immunological competence to neonatally thymectomized mice, modulates surface epitopes in patients with immune deficiencies and improves immunocompetence in man and animals.

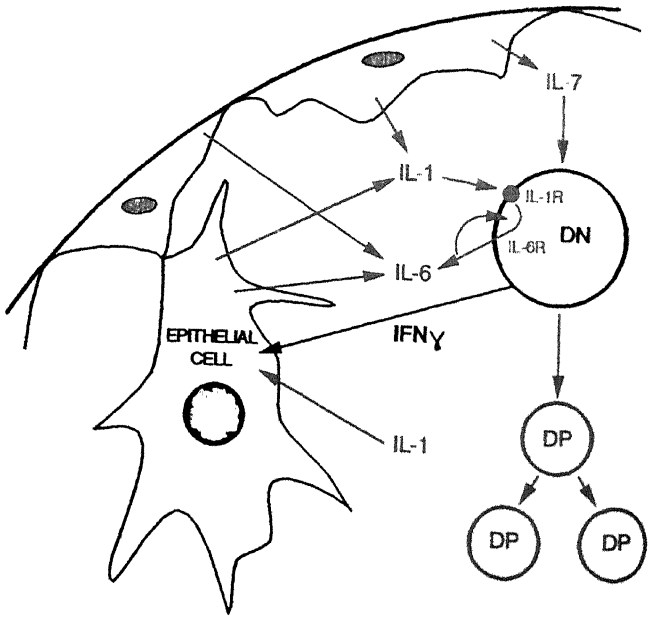
There are many other soluble factors in the thymic microenvironment, but cytokines have been shown (usually by in vitro methods) to be important singly or synergistically in thymocyte development. Their actions are very complex, and not yet fully understood. Interleukins IL-1, IL-2, IL-4 and IL-6 are secreted by thymocytes (as well as other cell types), and IL-1, IL-3, IL-4, IL-6 and IL-7 by the thymic epithelium. Cells bearing receptors for all of these cytokines, as well as for tumour necrosis factor α (TNFα), and colony stimulating factors for granulocytes and macrophages (GM-CSF, M-CSF or CSF-1) and γ-IFN, occur in the thymus.

NEUROENDOCRINE–THYMIC INTERACTIONS

All major hormones released from endocrine glands can influence thymic function and/or structure, and thymic factors often affect other endocrine organs. Many of these effects are mediated through the thymic microenvironment. Thymic epithelial cells have receptors for all of the sex steroid hormones (Grossman et al 1979; Pearce et al 1983), including a unique oestrogen receptor, corticosteroid receptors (Dardenne et al 1986), nuclear receptors for triiodothyronine (Villa Verde et al 1992) and low-affinity luteinizing hormone releasing hormone (LHRH) receptors. Receptors on thymocytes have been identified for growth hormone (Arrenbrecht 1974), corticosteroids (lower numbers than on epithelial cells), oxytocin (Elands et al 1990), and oestrogen (fewer than on epithelial cells) (Kawashima et al 1992).

Both gonadal steroids (except progesterone) and corticosteroids are well documented as affecting the thymus. Oestrogens can increase vascular permeability and downregulate Class II MHC and increase macrophage numbers, all of which would alter the thymic microenvironment function (Moreno & Zapata 1991). The numbers of thymocytes in early and late stages of differentiation are increased by high levels of oestrogens (Screpanti et al 1989).

Man is considered to be 'resistant' to the action of corticosteroids in the thymus (rats are 'sensitive'). In general, high levels of corticosteroids preferentially kill cortical thymocytes by causing apoptosis (reviewed in Kendall 1990) but low levels have a potentiating thymopoietic effect in the embryo (Ritter 1977). Corticosteroids also



9.31 Schema illustrating the proposed cytokine interactions within the thymus between subcapsular epitheliocytes and prothymocytes. DN: CD4−/CD8− cells; DP: CD4+/CD8+ cortical thymocytes. (Provided by M Kendall, Department of Pharmacology, UMDS, St Thomas's Campus, London.)

enhance the migration of prothymocytes into the thymus (Bomberger & Haar 1992). The weight of the thymus is increased by thyroid hormones, particularly exogenous T3, and in hyperthyroidism, while hypothyroidism decreases thymic weight. In rats, perinatal hypothyroidism causes a rise in thymic oxytocin content, but not in hypothyroid adults with atrophied thymuses. Some cortical epithelial cells synthesize oxytocin and vasopressin, and contain neurophysin (Geenen et al 1991), but immunostaining for oxytocin gives different reactions in thymus and brain (Geenen et al 1991). Oxytocin levels in the thymus are very high (Argiolas et al 1990a,b; Jeremovic et al 1990) and can be increased with corticosteroids such as dexamethasone.

Any effect of hypothalamic factors on thymic function is probably mediated via the hypophysis except for LHRH where there is histological, and some functional evidence for a direct action (Marchetti et al 1989). *Thymosin fraction 5* stimulates the secretion of LHRH from the hypothalamus (Rebar et al 1981). LHRH agonists cause thymic enlargement in rats and increase the levels of thymosin α1 (Ataya et al 1989).

The hypophysis is a target for the thymic hormones. Not all of them act in the same way, but release of many pituitary hormones can be induced in vitro either directly or via an influence on the hypothalamus. Antibodies against adrenocorticotrophic hormone (ACTH), growth hormone (GH), prolactin (PRL), follicle stimulating hormone (FSH), β-FSH, luteinizing hormone (LH), β-LH and other anterior hypophyseal hormones, give positive reactions with thymic epithelial cells, especially those of the medulla (Batanero et al 1992). PRL, GH (probably via IGF-1), and thyrotrophin releasing hormone (TRH) (through T3 action) increase thymulin secretion. The reaction is pleiotrophic and quite slow (over days in vitro). It is also effected by met-enkephalin and β-endorphin as well as adrenal and sex steroids (reviewed in Dardenne & Savino 1990). A rapid release of thymulin, however, is achieved by elevated physiological levels of ACTH in vitro and in vivo and its action is potentiated by glucocorticoids (Buckingham et al 1992).

Oestrogen (or testosterone metabolized to oestrogen) inhibits the release of thymic serum factors. The treatment of mice with estradiol reduced the levels of thymosin α1 (Allen et al 1984). These findings, however, are in contrast to those of Stimson and Hunter (1980) where estradiol treatment caused the appearance of thymus-dependent factors in the serum. Progesterone and estradiol applied over several days induced thymulin release from cultured thymic epithelial cells (Savino et al 1988). Earlier work, however, had shown a very complex picture of thymulin release during adrenalectomy and gonadectomy. Conflicting results could be due, in part, to the concentrations of steroids used. It can be seen from this rather heterogeneous list of experimental data that neuroendocrine interactions with the thymus are complex and, at present, difficult to simplify into a clear picture. However, it is patent that there is considerable interchange, and that extreme modulation of thymic function must be important in the control of immune function in general, through direct effects on T-cell maturation, and indirect effects on more subtle activities of the thymus on the immune system of the body.

CLINICAL ASPECTS OF THE THYMUS

Congenital anomalies have been summarized by Gray and Skandalakis (1990). Undescended thymus, accessory thymic bodies and the rare cysts of the third branchial pouch are of no clinical significance (except where thymectomy is indicated). Patients with thymic agenesis, aplasia and hypoplasia, as in the Di George (Cridu-chat) syndrome and severe combined immune deficiency disease, have reduced lymphocyte numbers, and early death from infection is common. Most cases are familial, with autosomal recessive genes.

In young children a large thymus may press on the trachea, causing attacks of respiratory stridor. Thymic tumours may also compress the trachea, oesophagus and large veins in the neck, causing hoarseness, cough, dysphagia and cyanosis. Thymomas may develop in one lobe of the thymus without affecting the other. Many of these patients have myasthenia gravis and other autoimmune conditions, too. Myasthenia gravis, a chronic autoimmune disease of adults (Castleman 1966), is a diminution in power of certain voluntary muscles for repetitive contraction. Although there may be more than one condition with these signs, myasthenia gravis is essentially an autoimmune disease in which acetylcholine (ACh) receptor proteins of neuromuscular junctions are attacked by auto-antibodies. Muscles commonly involved are levator palpebrae superioris (leading to ptosis) and extraocular muscles (leading to diplopia). Others in the face, jaws, neck and limbs may be involved and in severe cases the respiratory muscles. About 10% of Caucasian myasthenia cases have a thymoma and 50% have medullary follicular hyperplasia. These are predominantly young females under 40 years of age who have a strong HLA-B8-DR3 expression. Thymectomy in this latter group often results in improvements in their symptoms. In the absence of a thymoma, the onset of myasthenia gravis occurs after 40 years of age in patients with a HLA-B7-DR2 phenotype, except for a group in which weaknesses are restricted to eye and eyelid movements (Willcox 1989).

As described above, during neonatal and early postnatal life, the thymus is essential to the normal development of lymphoid tissues. Thymectomy at this stage leads to a progressively fatal condition, with hypoplasia of the peripheral lymphoid organs, wasting and an inability to mount an effective immune response. By puberty, when the main lymphoid tissues are fully developed, thymectomy is less debilitating, but a reduction in effective responses to novel antigens ultimately ensues.

(9.32–38)

Lymph nodes are encapsulated centres of lymphocyte differentiation and proliferation, belonging to the class of *secondary* or *peripheral* lymphoid tissue. Structurally they are small, oval or reniform bodies, 0.1–2.5 cm long, lying in the course of the lymphatic vessels. Each usually has a slight indentation on one side, the *hilum*, through which blood vessels enter and leave; an *efferent lymphatic vessel* also emerges. Several *afferent lymphatic vessels* enter peripherally. Lymph nodes have a highly cellular *cortex* and a *medulla* containing numerous, poorly demarcated cavities which represent a network of minute lymphatic channels through which lymph from the afferent lymphatics filters, to be collected at the hilum by the efferent lymphatic. The cortex is deficient at the hilum, where the medulla reaches the surface. Lymph nodes are particularly numerous in: the neck, mediastinum, posterior abdominal wall, abdominal mesenteries, pelvis and proximal regions of the limbs. By far the greatest number of these lie close to the viscera, especially in the mesenteries. Besides their immune functions in generating mature, primed B and T cells, lymph nodes add antibodies to the circulation, and also filter particles, including microbes from the lymph by the action of numerous phagocytic macrophages within their lymphatic spaces.

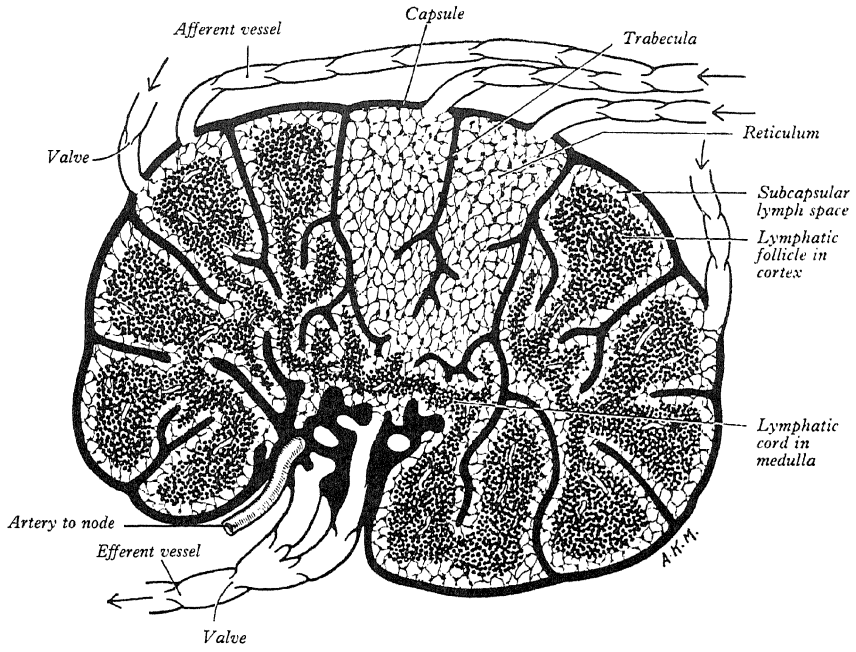
MICROSCOPIC STRUCTURE OF LYMPH NODES

A lymph node is essentially a continuous framework consisting of the capsule, trabeculae and the reticulum, with cells enmeshed in it.

Capsule and trabeculae. The capsule is composed mainly of collagen fibres, a few fibroblasts and elastin fibres, the latter being more numerous in the deeper layers. In some animals smooth myocytes occur but these are few in human nodes. From the capsule, trabeculae of dense connective tissue extend radially into the node's interior, continuous with the network of fine collagen (reticulin) fibres, the *reticulum*, which supports the lymphoid tissue. At the hilum, dense fibrous tissue may extend into the medulla, with an efferent lymphatic vessel embedded in it.

Reticulum. This network of fine collagen (reticulin) fibres and associated cells permeates the spaces enclosed in the capsule and trabeculae (**9.32**, 33) and supports the cell masses within them. Microscopically, the fibres are identifiable with reticulin stains (**9.34A**, B), which show how their bundles branch and interconnect, forming a very dense network in the cortex, although the germinal centres have fewer fibres. Bundles of these fibres, covered by endotheliocytes, criss-cross the sinuses, providing attachment for various cells, mostly macrophages and lymphocytes. Reticulin fibres with an associated proteoglycan matrix are apparently formed by cells indistinguishable from the fibroblasts, though various names (e.g. 'reticular cells') have been applied in the past.

Lymphatic channels. Lymph nodes are permeated by channels



9.32 Diagram of a lymph node (modified from Maximow & Bloom). In part of the diagram, lymphocytes have been omitted to show the reticulum. This diagram from an earlier source has been retained for historical interest; greater detail and more modern concepts are displayed in 9.33.

through which lymph percolates after its entry from the afferent vessels (9.37, 38A, C); because macrophages line channels or are entangled amongst the fibres crossing them, lymph is exposed to the action of these phagocytic cells, as well as to the activities of B and T lymphocytes adhering to endothelia.

Afferent vessels enter at many points on the periphery, branching to form a dense intracapsular plexus and then opening into the *subcapsular sinus*, a cavity peripheral to the whole cortex except at the hilum (9.33). From this sinus numerous radial *cortical sinuses* lead to the medulla, coalescing as larger *medullary sinuses*, which are in turn confluent at the hilum with the efferent vessel draining the node. All these spaces are lined by an endothelium which is continuous despite the constant passage of lymphocytes, macrophages and other cells through the walls of sinuses in both directions. Numerous trabeculae cross them, making their lumina almost labyrinthine and providing large areas for the attachment of various cell types in the spaces (Nopajaroonsri et al 1971; Luk et al 1973).

Lymphatic vasculature. Arteries and veins serving lymph nodes pass through the hilum, giving off straight branches which traverse the medulla and issue minor branches en route. On reaching the cortex, arteries form dense arcades of arterioles and capillaries in numerous anastomosing loops, eventually returning to highly branched venules and veins. Capillaries are especially profuse around the follicles, with fewer vessels within these structures (Herman et al 1973; Blau et al 1976); postcapillary venules are abundant in the paracortical zones, forming an important site of lymphatic migration (see below). The pattern of vascularization is altered when lymphocytes multiply in response to antigenic stimulation, and then the density of the capillary beds increases greatly (Herman et al 1972).

The structure of these blood vessels is normal except for the *postcapillary venules*, which are lined by tall cuboidal endothelial cells, between which colloids pass readily to perivascular spaces (Mikata & Niki 1971); they also allow extensive movement of lymphocytes from the bloodstream into the paracortex and probably also the reverse (Marchesi & Gowans 1964; Gowans & Knight 1964). The route of migration appears to be through tight junctions which part to allow the passage of cells (Schoefl 1972). Some veins may leave a node through its principal trabeculae and capsule, supplying these and the surrounding connective tissue.

Cells. Most of the cells in the reticulum are *B* and *T lymphocytes* but *macrophages* also occur, especially along the walls of sinuses and within germinal centres (see below). The distribution of lymphocytes varies in different regions. In the sinuses are some cells which are swept into lymph as it circulates through the node; in the cortex, cells are densely packed and may form isolated *lymphoid follicles* (nodules 9.35, 36). The number and isolation of follicles vary according to the prevailing antigenic stimulation. The follicular centre is composed of cells which are larger, less deeply staining and more rapidly dividing than those at its periphery. These areas are *germinal centres*, their cells being mainly *lymphoblasts* which, by prolific mitotic divisions, produce small lymphocytes which migrate outwards into the *mantle zones* surrounding the germinal centres. The cells pass from follicles into sinuses, which convey them across the medulla to the hilar efferent vessel.

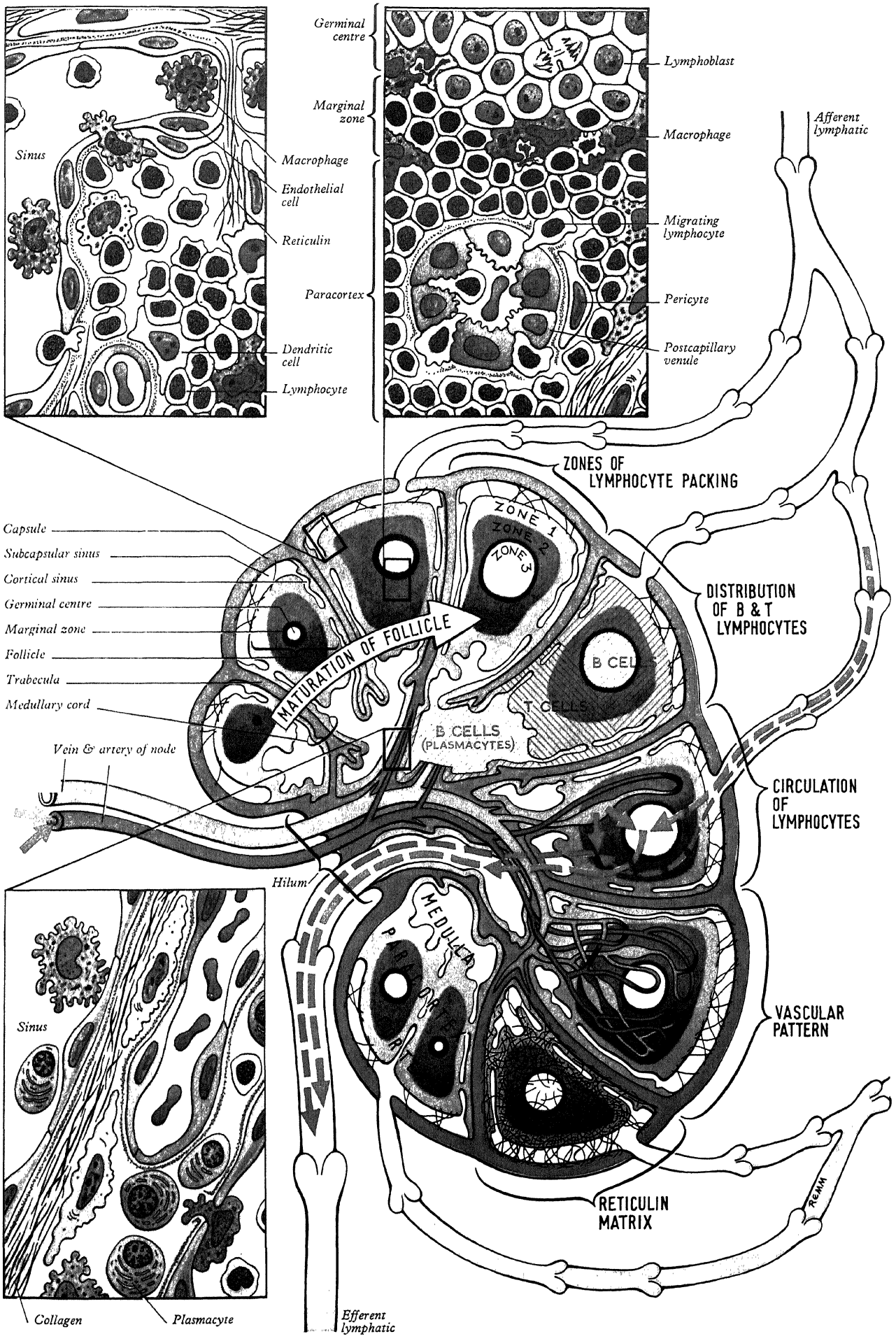
In the medulla, lymphocytes are much less densely packed, forming irregular branching *medullary cords* between which the reticulum is easily seen. Entangled cells include some macrophages, more numerous in the medulla, plasmacytes and a few granulocytes. Under antigenic stimulation, for example, when the node drains a site of infection or an immunization, the whole node **reacts** by an increase in size and vascularity. The number and size of germinal centres also increases, as lymphocytes and macrophages proliferate, and differentiation of numerous plasma cells occurs in the sinuses. For further details of lymphocyte biology, see pages 1405, 1422.

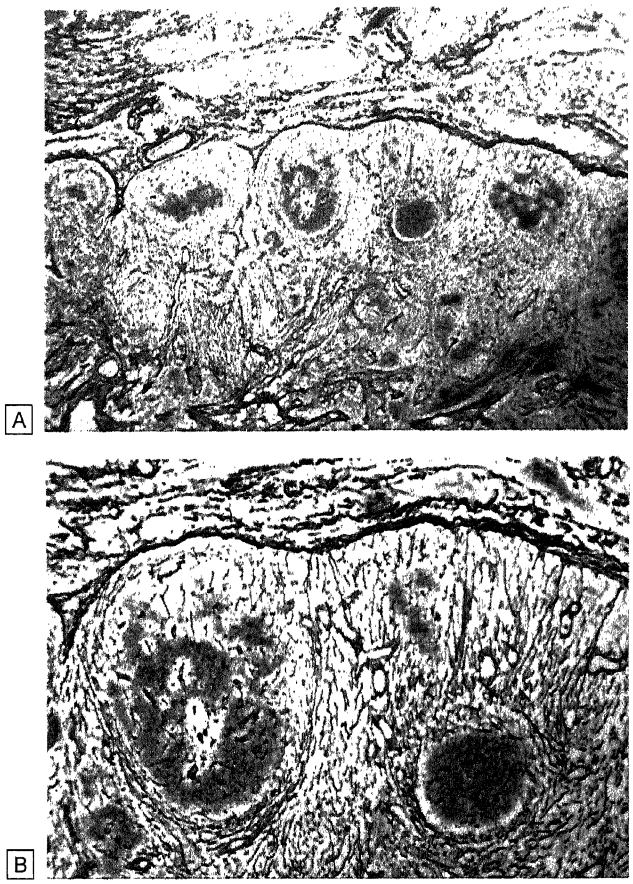
CELL ZONES IN LYMPH NODES

As stated above, the cells of lymph nodes are arranged in regions of different packing densities and of distinct cell types. Nopajaroonsri et al (1971) have suggested the division of the cortex into three zones indicating the packing density of its cells (9.33):

- *Zone 1* is a region of loosely packed cells, predominantly small lymphocytes, macrophages and occasional plasmacytes around the extreme periphery of follicles and extending centrally into the medullary cords;
- *Zone 2* is a denser region internal to zone 1, limited to cortical and paracortical areas and composed mainly of small lymphocytes and macrophages;

9.33 (*opposite*) Schema of the general architecture and cellular organization of a lymph node. Particular reference is made to the differential distribution of lymphatic spaces and cell masses. Coloured arrows ind the circulatory pathways of T and B lymphocytes. For details see text.


Germinal centre
Marginal zone
Paracortex
Lymphoblast
Macrophage
Migrating lymphocyte
Pericyte
Postcapillary venule
Afferent lymphatic
Sinus
Macrophage
Endothelial cell
Reticulin
Dendritic cell
Lymphocyte
ZONES OF LYMPHOCYTE PACKING
ZONE 1
ZONE 2
ZONE 3
MATURATION OF FOLLICLE
DISTRIBUTION OF B & T LYMPHOCYTES
B CELLS
T CELLS
B CELLS (PLASMACYTES)
Capsule
Subcapsular sinus
Cortical sinus
Germinal centre
Marginal zone
Follicle
Trabecula
Medullary cord
Vein & artery of node
CIRCULATION OF LYMPHOCYTES
Hilum
MEDULLA
VASCULAR PATTERN
RETICULIN MATRIX
Sinus
Collagen
Plasmacyte
Efferent lymphatic



9.34A, B Sections of a lymph node stained by the method of Glees and Marsden for reticulin. Note the heavy concentration of fibres in the capsule and trabeculae. A fine network permeates the rest of the node, with a concentric accumulation surrounding the cortical lymphatic follicles.

- *Zone 3* comprises the germinal centres of follicles, which are particularly prominent in antigenically stimulated lymph nodes; its cells include large lymphoblasts, some in mitosis, together with dendritic cells and macrophages.

Fibroblasts, reticulin fibres and other cells, mentioned below, appear in all zones. These zones may form a maturational sequence, lymphocytes arising by division in germinal centres (zone 3), passing to the dense zone 2, becoming smaller and finally migrating to zone 1, from which they may traverse the endothelium into the sinuses. But the sequence is complicated by immigration of other cells from lymphatic and haemal sources, and the precise relation between overall cellular architecture and maturation is not clear. In any case, though helpful for descriptive purposes, purely structural schemes also have to note the different functional subclasses of lymphocytes in such zones.

It is also possible to map the distribution of B and T lymphocytes within nodes by their immunohistological reactions to monoclonal antibodies (9.36A, B). Immunofluorescent staining shows that they occupy distinct territories. Immature B cells occur in the more peripheral parts of follicles, whereas T cells lie mainly between the germinal centres and the medulla, i.e. in the *paracortex* or *thymus-dependent zone*. Mature B cells (plasmacytes) exist mainly in medullary cords and sinuses, while some are also peripheral to the cortical follicles. The distribution of T lymphocytes is clear in animals with a congenital absence of the thymus (e.g. '*nude*' mice), which fail to develop a paracortex. Whether germinal centres contain T or B cells or both is debatable; possibly also cytological markers for their detection are only effective after the lymphocytes have left the germinal centres.

Other types of cell in lymph nodes

The different types of 'non-lymphocytic' cells have been considerably clarified by recent studies although certain details are as yet unclear. Following Steinman et al (1974) and other authors, we can distinguish the following: endothelial cells, fibroblasts, typical macrophages, follicular dendritic cells and paracortical dendritic cells ('interdigitating cells of the paracortex'); cells lining blood-vessel walls (smooth myocytes, pericytes) also occur, as do the terminals of non-myelinated nerve fibres. Endothelial cells lining the nodal sinuses appear typical in structure and, contrary to earlier views, do not show the phagocytic ability which had attracted terms such as 'endothelial macrophages', 'reticulo-endothelial cells', 'reticular cells' or 'littoral cells', although true macrophages do occur adhering to endothelium along the walls of sinuses. The endothelial walls appear to be of the discontinuous type, allowing the free passage of lymphocytes and macrophages. Fibroblasts ('reticular cells' of some authors) produce collagen including reticulin to form the nodal framework, i.e. the capsule, trabeculae and reticulum. Highly phagocytic macrophages (see p. 1415) are identifiable in the cortex within and between the follicles and elsewhere.

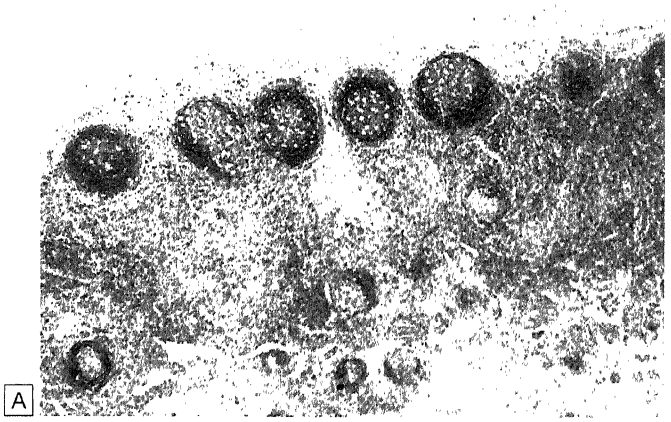
FUNCTIONS OF LYMPH NODES

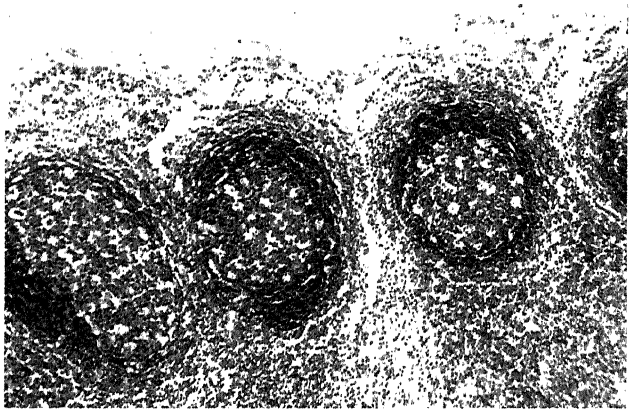
Lymphatic capillaries and larger lymphatic vessels returning excess tissue fluid to the venous system carry particulate materials of various kinds to the lymph nodes scattered along their course. The nature of these materials will vary with the region drained; areas rich in microbial flora, for example the alimentary tract, are a major potential route of entry of pathogenic organisms and debris into the circulation and of course any area of the body may supply microbes and debris of various kinds, particularly after damage or local infections. In the respiratory tract there is the additional problem of the removal of inhaled particles from the alveoli, which is carried out in part by macrophages re-entering the tissues and passing into the lymphatic pathways. Lymph nodes form a major protection against such materials and organisms, removing them by phagocytic activity and exposing them to a wide variety of powerful defensive actions carried out by lymphocytes resident within them or added to the population of defensive cells circulating in the lymph and blood. Lymph nodes respond dynamically to the presence of such materials and can modulate their activities and structure according to the demands put upon them.

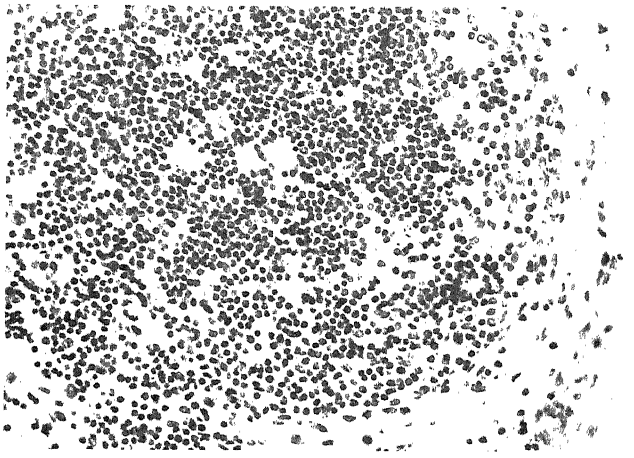
The essential roles of lymph nodes include:

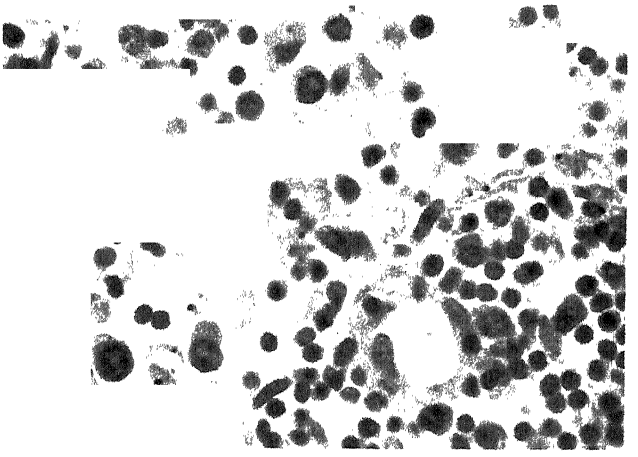
- the provision of a labyrinth of channels, of large volume and surface area, through which lymph slowly percolates
- the exposure of foreign material in the lymph to macrophages in nodal sinuses
- the trapping of antigens by different mononuclear phagocytes including dendritic types
- production of lymphocytes and a pool of stem cells able to become antibody-producing B lymphocytes and mature T lymphocytes
- interaction between APCs and lymphocytes to produce an immune response, both cell-mediated and humoral
- re-entry of blood-borne lymphocytes into lymphatic channels and thence to the haemal circulation
- humoral antibody production and addition to lymph and, via that route, to blood.

There have been a number of important advances in this area recently, based on cell labelling with antibodies against T- and B-cell markers, in situ hydridization detection of specific mRNAs, and culture methods. It appears that when T and B cells first enter the lymph node through the high endothelial venules in the paracortex, they initially come into contact with interdigitating antigen-presenting cells and undergo a process of selection and stimulation. The T cells mainly stay in the same area or enter the sinusoids to pass out of the lymph node through the efferent lymphatic and thus into the general circulation. The primed B cells, however, pass into the follicles, where they displace the existing small lymphocyte population, which are pushed to the perimeter of the follicle to create its mantle zone. The B cells then come to lie amongst the reticular network formed by the follicular dendritic cells (an antigen-



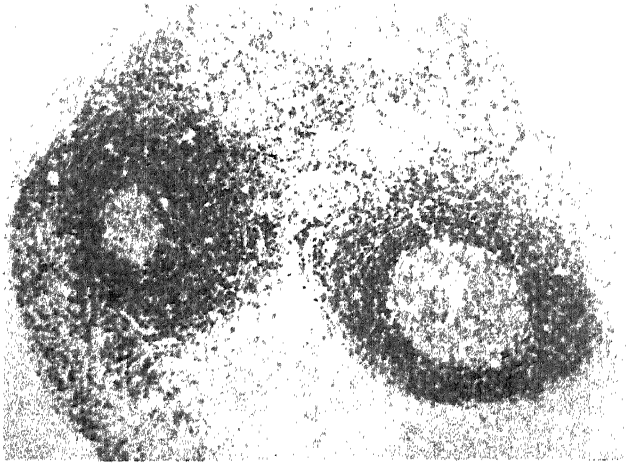


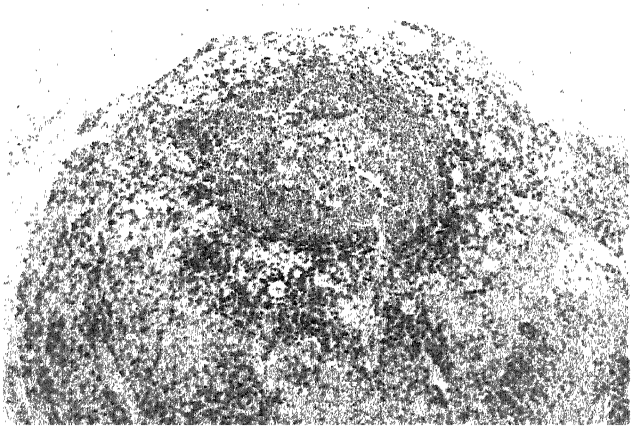




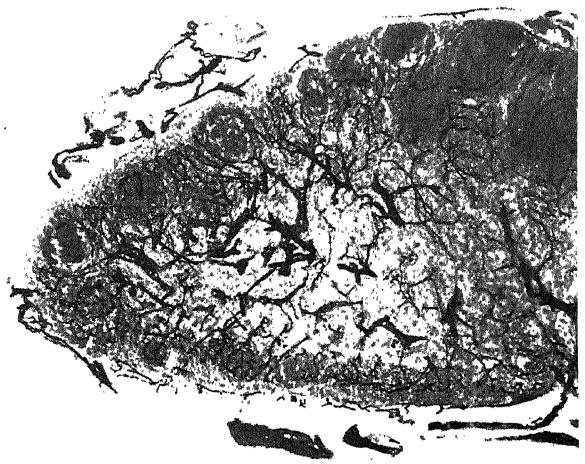
9.35A–D Sections of lymph nodes stained with haematoxylin and eosin. A Note the round cortical lymphatic follicles with their dense, dark periphery and pale germinal centres and the irregular medullary tissue. Very low power survey micrograph. B Low-power view of germinal centres showing the variation in cell density. C Higher-power view of the peripheral zone of a follicle showing the densely packed small lymphocytes. D Higher-power view of the medulla showing a variety of cell types including small and large lymphocytes and prominent rounded plasmacytes.

presenting cell, APC) which possess antigen on their surfaces (probably having endocytosed this elsewhere before reaching the node via the same route as the lymphocytes). Contact between the antigen and the B-cell surface receptors selectively triggers intense cell division in the B cells (which are then first termed centroblasts, and subsequently centrocytes); only those B cells which are able to mount defensive responses to the relevant antigens are stimulated to divide, forming clones of B cells with identical responses (see p. 1422, clonal theory). These activated B lymphocytes then move to the edge of the follicle, where they may express IgM as an initial response to stimulation, then pass into the sinusoids in which they move to the medulla of the node and thence into the circulation, or they may





9.36A, B Sections through lymph nodes showing different cells stained with the indirect antibody method (second antibody, HRP labelled). In A, lymphocytes of the B-cell class in two germinal centres are stained brown; in B, T cells are demonstrated chiefly in paracortical areas. (Provided by R Poston, Department of Histopathology, UMDS, Guy's Campus, London.)



9.37 Lymph node (guinea-pig) in section, after blood vessels have been injected with indian ink. Note the large vessels in the medulla, ramifying to form a capillary plexus in the cortex. (Provided by N Blau, UMDS, Guy's Campus, London.)

remain within the node as mature B cells (plasmacytes) secreting antibody. Those in the circulation can migrate out into the tissues through venules or capillary walls on demand, and also for normal immune surveillance.

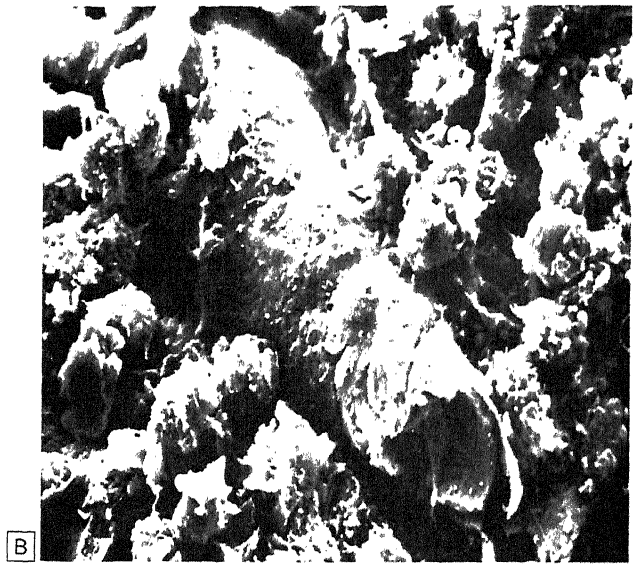
Haemal nodes

In human lymph nodes, erythrocytes may sometimes be found within sinuses. In some animals, small encapsulated lymphoid bodies occur in relation to the abdominal and thoracic viscera, where the sinuses are typically filled with blood, giving the whole structure a red colour; these are termed *haemal nodes* and appear to be more closely related to the blood-vascular than to the lymphatic system, since they lack afferent lymphatics (although they have a single efferent lymphatic vessel). Their structure has been described in detail by Turner (1969), Nopajaroonsri et al (1974).

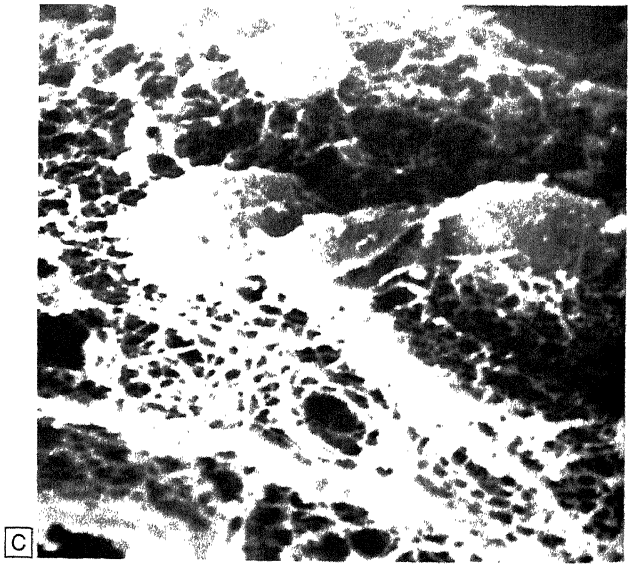
Fast and slow routes of blood circulation have been reported, the former through arterioles, capillaries and venules, the latter through a tortuous sinusoidal system. Specialized postcapillary venules with a high endothelium occur as in ordinary nodes. It is possible that haemal nodes are intermediate between lymph nodes and spleen, and perhaps a basis from which both have evolved. Their human incidence remains uncertain. In some animals, *haemolymph nodes* have been described, with a structure intermediate between that of the lymphatic and haemal nodes and with both lymphatic **and** vascular connections. Some consider them stages in the transformation of lymph into haemal nodes; others deny the existence of such intermediary structures.

Clinical anatomy of lymph nodes. Lymphatic vessels and nodes draining infected areas are liable to inflammation, resulting in acute or chronic lymphangitis and lymphadenitis. Chronic lymphangitis, with blocking of vessels by the escaped ova of a minute parasitic worm, *Wuchereria bancrofti*, is the cause of elephantiasis (filariasis), typified by enormous thickening and reduplication of skin, frequently in the lower limbs and scrotum. Blockage of lymphatic vessels may also result from the spread of neoplasms or widespread surgical removal of nodes. Neoplastic cells may spread by minute emboli or may grow along lymphatic vessels in solid masses. Removals of tumours are therefore planned to take away **in one mass** the tumour,

A



B



C

9.38A–C Scanning electron micrographs of the cut surface of a lymph node (guinea-pig). A Low-power micrograph of the outer cortex showing the capsule (right) together with the subcapsular sinus traversed by reticular fibres. Part of a germinal centre is visible on the left. Magnification ×400. B Medium-power micrograph of part of a germinal centre, showing lymphocytes clustered around a capillary. Magnification ×2000. C High-power micrograph of the wall of the subcapsular sinus, showing the fine network of reticular fibres with some attached cells. Magnification ×6000.

the intervening vessels and local nodes. It is important to note that lymphatic vessels from a region may not drain to the local lymph nodes, but to those more remote, often making operative removal difficult if not impractical.

INTRODUCTION

The spleen consists of a large encapsulated mass of vascular and lymphoid tissue situated in the upper left posterior region of the abdominal cavity between the fundus of the stomach and the diaphragm (**10**.114, **12**.77A, 97). After fixation in situ (**9**.39), its shape varies from a slightly curved wedge to a tetrahedron, depending on how much it was indented by the neighbouring colon at the time of death, the shape of the spleen being largely moulded by the surrounding structures. Its long axis lies approximately in the plane of the tenth rib, its posterior border being about 4 cm from the mid-dorsal line at the level of the tenth thoracic vertebral spine; its anterior border reaches the mid-axillary line.

The size and weight of the spleen vary with age, with the individual and in the same individual under different conditions. In the adult it is usually about 12 cm long, 7 cm broad and 3–4 cm wide. It tends to diminish in size and weight in older people. Its average adult weight is about 150 g (normal range: 80–300 g, largely reflecting its blood content).

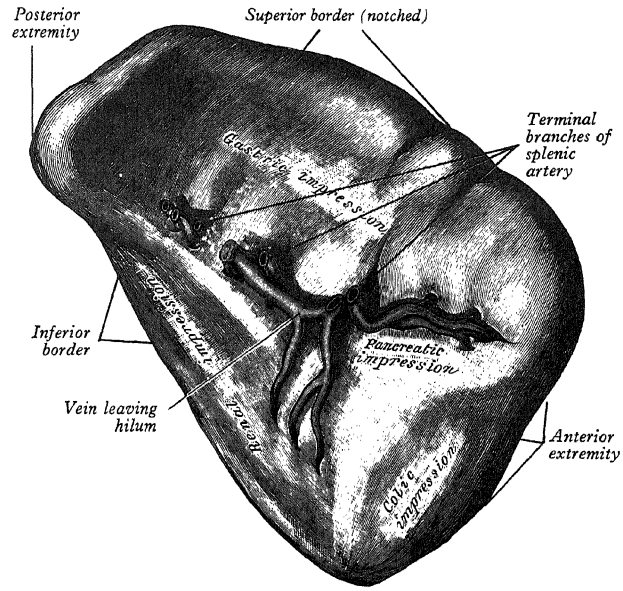
The spleen has two major functions: the removal of particulate material including ageing erythrocytes from the circulation, and the provision of lymphocytes and antibodies as part of the body's system of secondary lymphoid tissues. Both of these activities are shared with other organs in the body, so the spleen is not essential to survival, although its removal diminishes the body's defence against disease.

TOPOGRAPHY AND RELATIONS OF THE SPLEEN

The spleen has diaphragmatic and visceral surfaces, superior and anterior borders and inferior and posterior extremities. The *diaphragmatic surface*, which is convex and smooth, faces posterosuperiorly and to the left, except at its posterior edge which faces slightly medial. It is related to the abdominal surface of the diaphragm which separates it from the lowest part of the left lung and pleura and the ninth to eleventh left ribs. The pleural costodiaphragmatic recess extends down as far as its inferior border. The *visceral surface* (**9**.39), facing the abdominal cavity, presents gastric, renal, pancreatic and colic impressions. The *gastric impression*, directed anteromedially and upwards, is broad and concave where the spleen abuts on to the posterior aspect of the stomach, from which it is separated by a recess of the greater sac. Near the inferior limit of the spleen is the hilum, a long fissure pierced by several irregular apertures through which vessels and nerves of the spleen enter and leave. The *renal impression*, which is slightly concave, is located on the lowest part of the visceral surface and is separated from the gastric impression above by a raised margin. It faces inferomedially and slightly backwards, being related to the upper and lateral area of the anterior surface of the left kidney and sometimes to the superior pole of the left suprarenal gland. The *colic impression*, at the extreme lateral end of the spleen, is usually flat and is related to the left colic flexure and phrenicocolic ligament (**12**.97). The *pancreatic impression*, small when present, is situated between the colic impression and the lateral part of the hilum; it is related to the tail of the pancreas which lies in the lienorenal ligament (**12**.77A).

The *superior border*, separating the diaphragmatic surface from the gastric impression, is usually convex and, near its lateral end, has one or two notches indicating the lobulated form of the spleen in early fetal life (p. 328). The *inferior border* separates the renal impression from the diaphragmatic surface and lies between the diaphragm and the upper part of the left kidney's lateral border. More blunt and rounded than the superior border, it corresponds in position to the eleventh rib's lower margin.

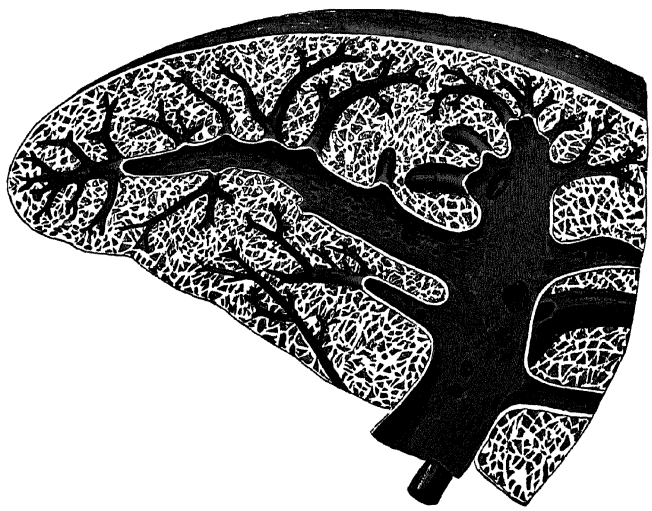
The *posterior extremity* usually faces the rounded vertebral column. The anterior extremity is more expanded and commonly forms a margin connecting the lateral ends of the upper and lower borders. It is related to the left colic flexure and to the phrenicocolic ligament.



9.39 The visceral surface of the spleen.

The spleen is almost entirely covered by peritoneum, which adheres firmly to its capsule. Recesses of the greater sac separate it from the stomach and left kidney. It develops in the upper dorsal mesogastrium (**3**.76), remaining connected to the posterior abdominal wall and stomach by two folds of peritoneum, respectively the lienorenal ligament and the gastrosplenic ligament. The *lienorenal ligament* is derived from peritoneum where the wall of the general peritoneal cavity meets the omental bursa between the left kidney and spleen; the splenic vessels lie between its layers (**12**.77A). The *gastrosplenic ligament* also has two layers, formed by the meeting of the walls of the greater sac and the omental bursa between spleen and stomach (**12**.77A); the short gastric and left gastro-epiploic branches of the splenic artery pass between its layers. Most laterally the spleen is in contact with the phrenicocolic ligament.

The spleen is also covered externally by a series of connective tissue bars (trabeculae); they ramify throughout the whole structure to create a fibrous skeleton supporting its delicate tissues, which include both lymphoid tissues (white pulp) and extensive areas of



9.40 Transverse section through the spleen, showing the trabecular tissue and the splenic vein and its tributaries. From the first edition of *Gray's Anatomy* (1858).

blood-filled tissue (red pulp). In the living the spleen is soft and friable, and is dark purple because of the considerable amount of blood within its substance.

Near the spleen, especially within the gastrosplenic ligament and greater omentum, small encapsulated nodules of splenic tissue may occur, isolated or connected to the spleen by thin bands of similar tissue. Such *accessory spleens* or *spleniculi* may be numerous and widely scattered in the abdomen. The spleen may retain its fetal lobulated form or show deep notches on its diaphragmatic surface and inferior border in addition to those usually present on its superior border.

Surface anatomy. The position of the spleen in the living can be assessed by percussion; the dull area extends over the ninth to eleventh ribs in vertical extent and should not go forward beyond the midaxillary line. The normal spleen is not palpable.

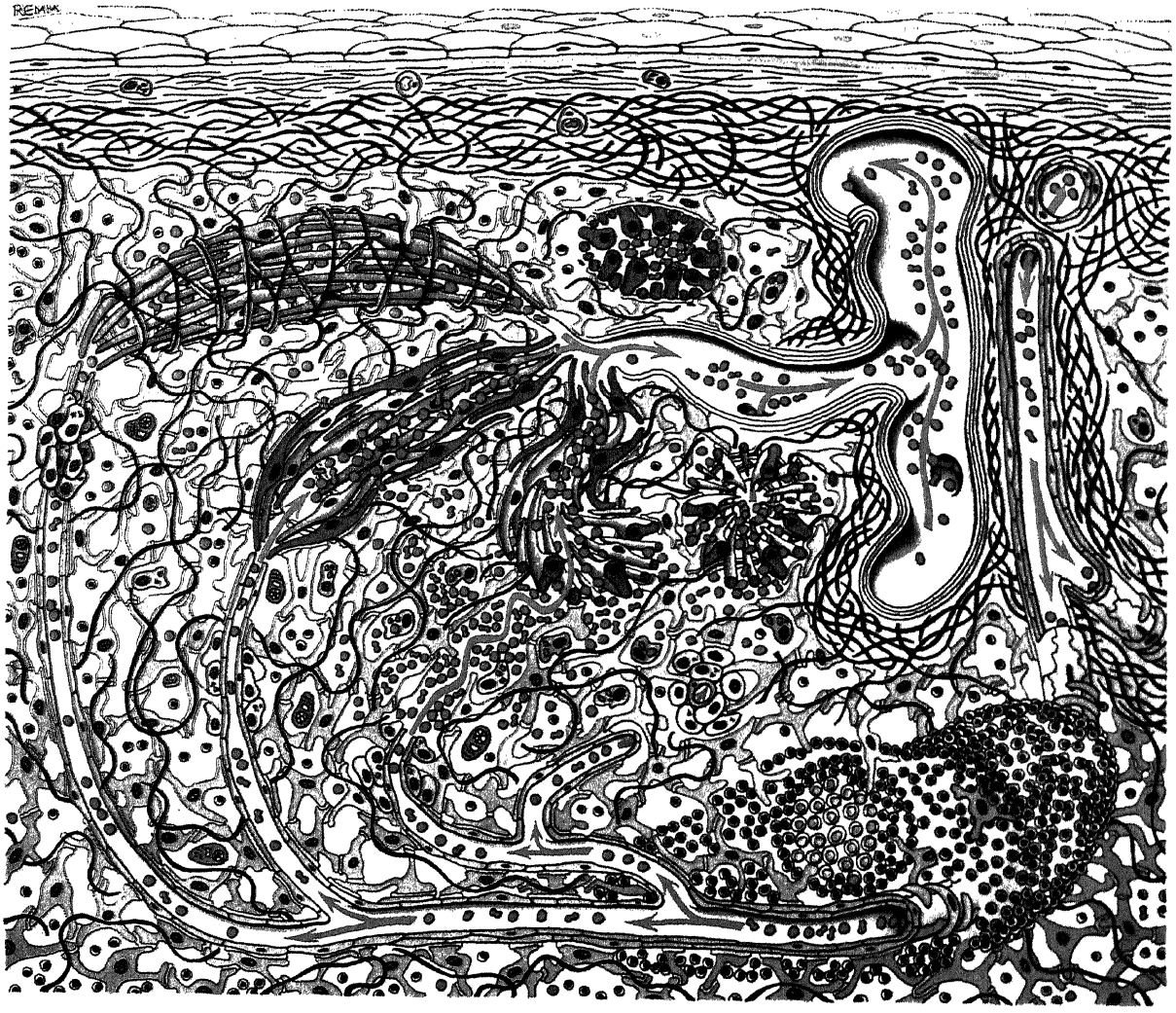
Vessels and nerves of the spleen

Arteries. The spleen receives its blood from the splenic artery, a large tortuous branch of the coeliac artery. After giving off various minor branches to the pancreas and stomach, this vessel divides in the lienorenal ligament shortly before reaching the spleen into two or three main branches from which four, five or more *segmental branches* enter the spleen's hilum to supply a territory within it termed a *splenic segment* (see below). Within each segment, the artery ramifies in the trabeculae to supply the parenchyma and capsule of the spleen. The pathway of blood beyond this point is considered later (p. 1551).

Veins (9.40). The minor veins pass from the red pulp of the spleen into the trabeculae, and thence into segmental veins running alongside the segmental arteries. On leaving the hilum, they continue in company with the arterial branches, draining into the main splenic vein in the lienorenal ligament. After receiving venous tributaries from various sources, the splenic vein usually drains directly into the hepatic portal vein (p. 1603).

Lymphatics. These drain along the splenic trabeculae to pass out of the hilum into the lymphatic vessels accompanying the splenic artery and vein. They take splenic lymph to the pancreaticosplenic and coeliac nodes (p. 1618, 1619).

Nerves. The coeliac sympathetic plexus gives off nerve fibres which pass along the splenic artery and its branches as a surface plexus, to enter the hilum and run with the segmental arteries and their branches. These fibres appear to be mainly noradrenergic vasomotor, concerned with the regulation of blood flow through the spleen; adrenergic agonists inhibit the concentration of red cells in the splenic blood (plasma skimming, see below) indicating that sympathetic activity causes an increase in the 'fast' circulation of the spleen as opposed to slow filtration (Reilly 1985). The presence of other neural connections has not been demonstrated.



9.41 The main features of splenic structure; the various elements are not drawn to scale, to enable representation on a single diagram. Note the capsule, trabeculae, reticular fibres and cells, the perivascular lymphatic aggregation (white pulp), and the ellipsoids, cell cords and venous sinusoids of the red pulp. The 'open' and 'closed' theories of splenic circulation are illustrated. The venous sinusoids are shown in two states: (1) with their lining of 'stave' cells (bright blue) in close apposition, (2) with intercellular gaps (these have been over-emphasized for clarity). Consult text for further details.

Splenic vascular segmentation

There is evidence (Dreyer & Budtz-Olson 1952) that the human spleen, like that in other species, consists of separate 'segments' or 'compartments', each served by a hilar branch of the main splenic artery and splenic vein (Braithwaite & Adams 1957). Adjacent compartments, it is claimed, are connected by intersegmental veins; a congested compartment can thus pass excess blood to those adjacent (see below) but, when blood flow is not excessive, splenic segments may act as separate units. (For a review of splenic segmentation since first proposed by Kyber 1870, consult Gupta et al 1976; Treutner et al 1993.) Gupta et al studied corrosion casts of 50 adult human spleens. In 42 of these (84%) only two segments existed (superior and inferior); in 8 (16%) three segments (superior, intermediate and inferior) were demonstrated. There was no clear anastomosis between segments. A comparable segmental arrangement of splenic veins was described by Fuld and Irwin (1954). The occurrence of only two or three segments (arterial or venous) was supported by many investigators quoted by Gupta et al; but this does not accord with the usual description of splenic arteries dividing into five or more major branches in the lienorenal ligament before even entering the hilum: no explanation of this discrepancy is advanced by these authors.

SPLENIC MICROSTRUCTURE (9.41–45)

Microscopically, the internal mass (parenchyma) of the spleen consists of two major components, known as *white pulp* and *red pulp*, denoting their appearance when the freshly excised spleen is transected. The white pulp is composed of lymphoid tissue in which B and T lymphocytes can mature and proliferate under antigenic stimulation. The red pulp is a unique filtration device which enables macrophages in the spleen to extract particulates from the blood as it perfuses this organ. Red pulp is composed of a complex system of interconnected spaces inhabited by large numbers of phagocytic macrophages. These cells remove and dismantle effete red blood cells, micro-organisms, cellular debris and other particulates from the circulation. At the junction of white and red pulp is a narrow *marginal zone*, an area important in establishing immune responses and other aspects of splenic biology.

Fibrous framework of the spleen

The serosa of the peritoneum covers the entire organ except at its hilum and along the lines of reflexion of the lienorenal and gastrosplenic ligaments. Deep to this layer is the connective tissue *capsule*, a continuous layer about 1.5 mm thick, rich in collagen but also containing some elastin fibres. The capsule has an outer and an inner lamina in which the directions of collagen fibres differ (Faller 1985), presumably increasing its strength. From the capsule numerous trabeculae extend into the substance of the spleen, branching within it to form a supportive framework. The largest trabeculae enter at the hilum and ensheathe the splenic vessels and nerves, dividing into branches in the splenic pulp (**9.40, 41**). Like the capsule, trabeculae are composed of dense irregular connective fibres, rich in collagen and elastin. In many mammals, for example the cat or the horse, both capsule and trabeculae contain many smooth muscle cells which enable the spleen to contract on autonomic stimulation to expel its considerable quantity of stored blood into the general circulation. Such spleens are termed *storage spleens* (Weiss 1990). The human spleen lacks this potential, and its functions are related primarily to protection (a *defence spleen*); there is little smooth muscle, and the contraction and distension of the spleen are attributable to the effects of constriction or dilation of its inflow and outflow vessels which alter the volume of blood in the organ. An increase of intrasplenic blood pressure distends the spleen and stretches the elastic fibres, while contraction is due to their recoil when the pressure drops. Within the spleen, branching trabeculae are continuous with a delicate network of fine collagen (reticulin) fibres pervading both the white and red pulp, laid down by numerous fibroblasts which are present in its meshes (see also below).

White pulp

Within the spleen, the branches of the splenic artery radiate out from the hilum within trabeculae, ramifying a number of times and narrowing to arteriolar dimensions. In their terminal few millimetres, their connective tissue adventitia is replaced by a sheath of T lymphocytes, the *periarteriolar* (or *periarterial*) *lymphatic sheath*. This sheath is enlarged in places by *lymphoid follicles* (Malpighian bodies), aggregations of B lymphocytes visible to the naked eye on the freshly cut surface as white semi-opaque dots 0.25–1 mm in diameter, which contrast with the surrounding deep reddish-purple of the red pulp. Follicles are usually situated near the terminal branches of the arteriole, or at a larger branching point, and typically protrude to one side of the vessel. Like the periarteriolar sheaths, follicles are centres of lymphocyte proliferation as well as aggregation, and when antigenically stimulated, they develop germinal centres, as also in lymph nodes and nodules. The germinal centres regress when the infection subsides. Follicles also atrophy with increasing age and may be absent in the very old. The whole white pulp is supported by a network of fine collagen fibres interspersed with fibroblasts. Within the follicle, arterioles form a series of lateral terminal branches, often forming a series of parallel arterioles (called penicilli, alluding to their resemblance to the penicillium mould).

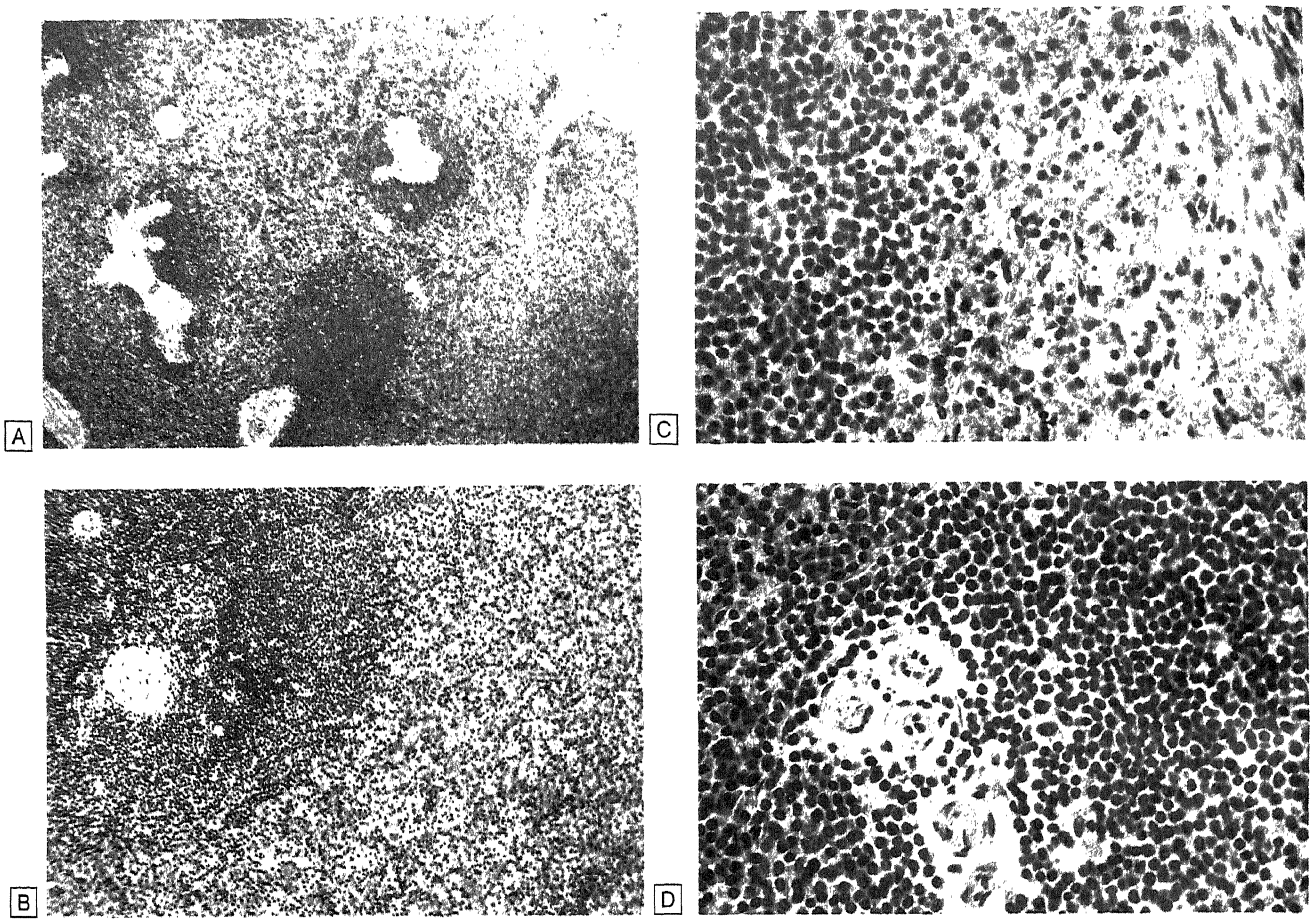
Red pulp (9.41, 43–45)

The red pulp constitutes the majority (about 75%) of the total splenic volume. Within it lie large numbers of venous sinuses draining into tributaries of the major splenic veins. The sinuses are separated from each other by a fibrocellular network, the *reticulum*, formed by numerous fibroblasts (*reticular cells*) and small bundles of delicate collagen fibres, in the meshes of which lie splenic macrophages. Seen in two-dimensional sections, these intersinusal regions appear as strips of tissue, *splenic cords* (of Billroth), alternating with splenic sinuses (**9.41, 44, 45**) although in reality they form a three-dimensional continuum around the venous spaces. To understand the organization of the red pulp some details of the sinuses and of the intersinusal reticulum are required, as follows.

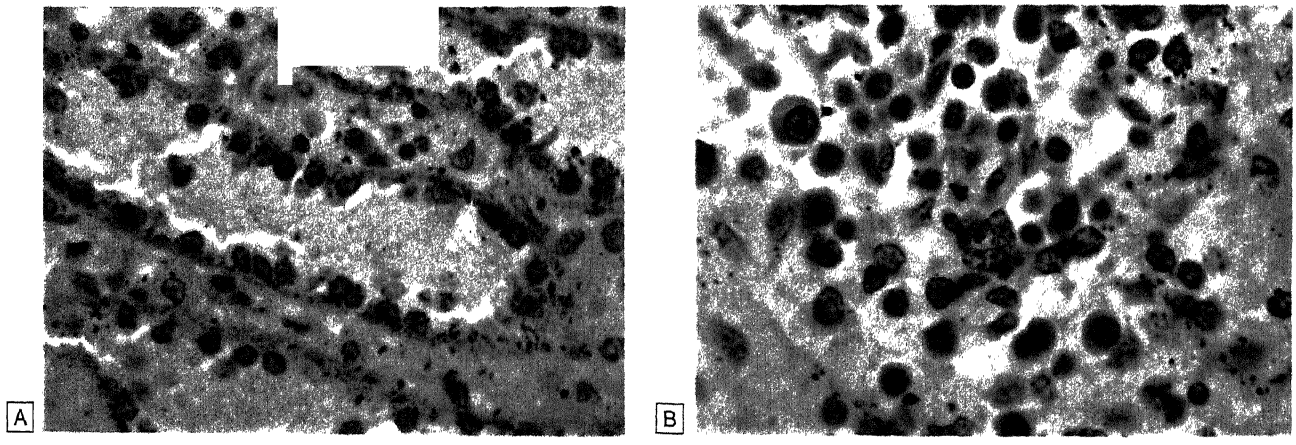
Venous sinuses. These are elongated ovoid vessels about 50 μm in diameter, lined by a characteristic 'incomplete' endothelium unique to the spleen. The endothelial cells are long and narrow, aligned with the long axis of the venous sinus (for this reason they are often called *stave cells*, reminiscent of planks in a barrel (**9.41, 44, 45**)); along their length they are attached at intervals to their neighbours by short stretches of intercellular junctions (tight and adhering) which alternate with intercellular slits through which blood can pass. The luminal and external surfaces of the cells bear short irregular microvilli, and numerous endocytic/exocytic vesicles are formed at both surfaces. Internally the endothelial cells possess a well-organized cytoskeleton, with longitudinal bundles of vimentin and of actin and myosin which probably determine their elongated shape, and could actively modulate the form of the cell to alter the sizes of the slits in the sinus walls, thus regulating the passage of blood through the intercellular gaps (see Chen & Weiss 1972, 1973).

The endothelial cells are mildly phagocytic (as are others of this type elsewhere in the body) but do not appear to contribute strongly to the uptake of particles in the spleen. A perforated, discontinuous basal lamina is present on the aspect of the sinus facing away from the lumen. The presence of slits between the endothelial cells allows blood cells to slowly squeeze from the surrounding splenic cords into the lumen of the sinus, the cells distorting considerably in the process (see below). The sinuses are supported externally by circumferential and longitudinal reticulin fibres, which are connected to the fibrous reticulum of the splenic cordal tissue around them.

Reticular tissue of the splenic cords. Around the sinuses the network of collagen fibres bears a population of large, stellate fibroblasts, the reticular cells. The extensions of these cells are flattened and leaflike, and help to divide the reticular space into a series of defined loculi which contain macrophages. These cells synthesize the matrix components of the reticulum, including the collagen fibres and various proteoglycans. Blood released into the reticular space from the ends of capillaries trickles through these spaces, receiving the phagocytic attentions of macrophages which are able to remove particulates from blood. Under conditions of heavy loading, for example when there are many damaged erythrocytes in the circulation to be removed by splenic macrophages, the reticular cells proliferate and increase the size of the red pulp considerably, thus causing enlargement of the whole spleen, and in extreme cases, splenomegaly (see over).



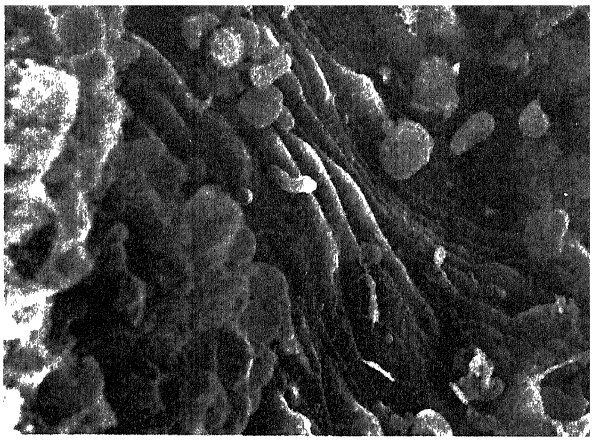
9.42 Sections of human spleen stained with haematoxylin and eosin. (Supplied by D R Turner of the Department of Pathology, Guy's Hospital Medical School.)
A, B Survey photographs at low power showing the general contrast between the white pulp (perivascular lymphatic aggregates, stained blue) and the red pulp (venous sinusoids and intervening cellular cords, stained reddish purple). C High-power view of the junctional (marginal) zone between the densely packed lymphocytes of the white pulp and the blood-filled sinusoids and cell cords of the red pulp. D A group of small (penicillar) arteries ensheathed by densely packed small lymphocytes.



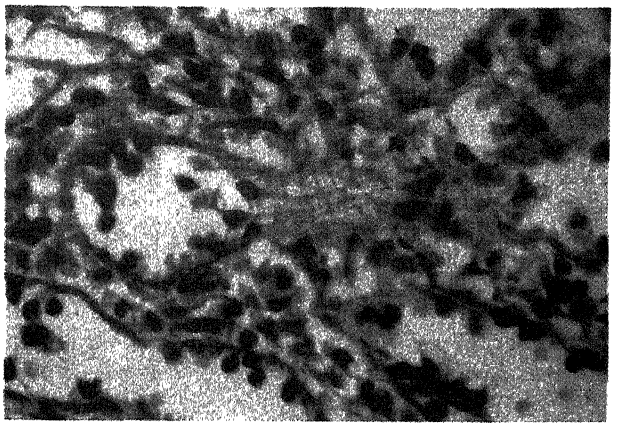
9.43 Section of monkey spleen following intravascular perfusion with a suspension of carbon particles followed later by perfusion fixation, stained by Weigert's haematoxylin and Van Gieson's stain.
A Showing empty, dilated venous sinusoids and intervening cell cords. The 'stave' cells lining the sinusoids are prominent. B High-power view of the cellular region between venous sinusoids. The cell types seen include reticular macrophages with carbon particles in their cytoplasm, small and large lymphocytes and a number of prominent rounded plasma cells.

Marginal zone

At the interface between the white and red pulp is the marginal zone, a region of great importance to the biology of the spleen. Here the lymphocytes are more loosely arranged than in the white pulp, and are held in a dense network of fine collagen fibres, intermingled with reticular cells. The arterioles leaving the white pulp often spread within the marginal zone before terminating, frequently bifurcating



9.44 Scanning electron micrograph of a splenic sinusoid (monkey) to show endothelial cells and associated tissue. Magnification × 1000.



9.45 A splenic sinusoid sectioned tangentially to show parallel strap-like endotheliocytes and surrounding tissue. Gomori's method. Magnification × 600.

immediately before this point. Close to their ends, where the lumen is much narrowed, these vessels are surrounded by a small aggregation of macrophages, the *periarteriolar macrophage sheath* (of Schweigger-Seidel) or *ellipsoid*. Ellipsoids are well developed in some mammals, for example the pig, cat and dog, but not in humans. The marginal zone is a region where blood is delivered into the red pulp, and also where many lymphocytes leave the circulation to migrate into their respective T- and B-lymphocyte areas of the white pulp. In some species there is an extensive slitlike *marginal sinus* between the white pulp and marginal zone, into which many arterioles discharge, and which are in continuity with the venous sinuses. In humans this seems to be less conspicuous although arguably present (see Groom & Schmidt 1990).

Splenic microcirculation (9.41)

The circulation of blood within the spleen has long been a subject for dispute, partly because of the complexity of the vascular channels, but also because of conflicting experimental evidence in different species (Knisely 1936; MacKenzie et al 1941; Snook 1950; Peck & Hoerr 1951; Lewis 1957a; Wennberg & Weiss 1969; Chen & Weiss 1972, 1973). Before discussing the present consensus, it is necessary to clarify the vascular anatomy.

The large, tortuous splenic artery, before reaching the spleen, divides in the lienorenal ligament into as many as five or more rami entering the hilum to ramify in the trabeculae throughout the organ. Likewise, the splenic vein forms in the ligament from an equal number of tributaries emerging from the hilum. As already noted, small arteries tapering to arterioles pass through the white pulp then turn abruptly to form penicillar branches which, after a course of about 0.5 mm, pass out of the white pulp into the marginal zone and red pulp. The passage of blood through the vascular compartments between the arterioles and splenic veins is referred to collectively as the *intermediate circulation* of the spleen. By a number of routes (see below), blood is passed to the venous sinuses from which it enters venules leading to small veins (the latter running within trabeculae) and thence into larger veins draining the spleen at its hilum.

Open and closed splenic circulations. Two schools of thought arose from the 1930s onwards concerning whether blood passed from the arterioles (or their terminal capillaries) directly into the venous sinuses (a closed circulation) or was instead discharged into a network of spaces in the splenic cords before entering the sinuses through the minute slits in their walls (an open circulation). Recently, various observations using a number of complementary techniques (reviewed by Weiss 1990; Groom & Schmidt 1990) on human and animal spleens have helped to resolve this issue. Measurement of the transit time of isotopically labelled blood through the cat spleen has shown that there are three distinct velocity components, fast, intermediate and slow (see Levesque & Groom 1976). About 90% of the blood passes through very rapidly, in a few seconds, as might be expected of a closed circulation through a capillary bed. About 9.6% takes minutes to pass through, and a small amount (1.6%) takes an hour or more.

Microscopic visualization of the circulation in the transilluminated rat spleen (MacDonald et al 1987), and anatomical analysis based on electron microscopy and micro-corrosion casts of human and animal spleens indicate a likely structural basis for these three rates of flow (Groom & Schmidt 1990). The rapid transit probably occurs through a proportion of arterioles which connect directly to the ends of sinuses, i.e. a 'closed' circulation, presenting little resistance to the passage of blood. Also in this category may be blood which is discharged from arterioles or capillaries close to those sinuses which have open ends, for example in the perimarginal sinus, and thus present relatively little resistance to blood flow although appearing to be part of the anatomically 'open' circulation. The intermediate circulation appears to reflect the presence of an anatomically and physiologically **open** circulation, in which blood percolates slowly through the reticular tissue of the splenic cords and filters through slits in the sinus walls before joining the majority of the blood flow; this process exposes the blood to maximal contact with splenic macrophages and is likely to be the period when removal of particles and effete red cells occurs. Finally, the slow circulation is thought to involve temporary adhesive contacts between blood cells and splenic cordal cells, since the passage of plasma is not slowed in the same way. Red cells, leucocytes and platelets can be sequestered in the spleen by such actions, sometimes for considerable periods, and of course some of these adhesive events are preliminary to the removal of damaged or aged cells by macrophagic phagocytosis.

The total volume of blood in the intermediate and slow circulation greatly exceeds that of the fast transit because of the relatively much greater volume of the splenic cords compared with the blood in the arteries, sinusoids and veins. The retarding effect on blood cells exceeds that on the flow of plasma so that the concentration of red cells (the haematocrit) within the spleen is about twice that of the general circulation and the number of reticulocytes is especially high; numerous platelets are also sequestered, but can be released into the circulation on demand.

It must be added that these views of splenic circulation are not entirely agreed between investigators and further clarification is to be expected. It is also probable that the proportions of blood flow

along these different routes vary with local changes in blood pressure within the spleen and in the general circulation.

FUNCTIONS OF THE SPLEEN

The spleen is essentially concerned with phagocytosis, immune responses, cytopoiesis and blood cell storage. In the fetus it is also an important site of haemopoiesis, and postnatally it may become haemopoietic in certain pathological conditions. However, although of great importance to the defence of the body, it is not absolutely essential since many of its functions can be assumed by the liver and by other lymphoid tissues if the spleen is removed.

Phagocytosis

Splenic macrophages constitute a large part of the mononuclear phagocytic system of the body (p. 1414). They are highly phagocytic cells distributed mainly in the splenic cords and marginal zones, where they are attached to reticulin fibres and neighbouring reticular cells. They can ingest particulate matter, micro-organisms and aged blood cells, especially erythrocytes and platelets. The processing of ageing or damaged red cells is especially important, as the spleen is the major site of their removal from the circulation. Within splenic macrophages, all stages of erythrophagocytosis, from disintegrating erythryocytes to granules of haemosiderin, can be discerned (Chen & Weiss 1972). Bilirubin, an end-product of haemoglobin catabolism, is conveyed in the bloodstream to the liver for excretion and the iron is largely re-used by bone marrow. Amino acids from the hydrolysis of globin are returned to the amino acid pool of the body. Splenic macrophages are also important in removing microbes and cellular debris from the circulation and their lysosomes possess many powerful enzymes which can hydrolyse or oxidize these bodies, particularly when the macrophages are activated by cytokines during immune responses (p. 1418) or the objects to be phagocytosed have been coated with antibodies.

How the macrophages recognize ageing red cells prior to phagocytic removal is an intriguing puzzle. Current evidence indicates that as erythrocytes get older, hitherto protected antigens in their surfaces become exposed to auto-antibodies in the circulation, and that the macrophages bind the antibodies which initiate phagocytosis (see also p. 1415).

Pitting of red cells

There is evidence that abnormal red cell inclusion such as Heinz bodies (intracellular masses of altered haemoglobin) can be removed from erythrocytes during their passage through the spleen without cell lysis. Exactly how this is done is not known. It has been suggested that such bodies might be squeezed out of the cell as it passes through the extremely narrow slits between sinusal stave cells, although how this could occur without red cell lysis is not clear.

Immune responses

Like other lymphoid organs, the spleen contains B and T lymphocytes in its white pulp and elsewhere, and also various antigen-presenting cells within the follicles and periarterial sheaths. It is, therefore, a site of antigen presentation by dendritic cells and the initiation of T- and B-cell activities involved in humoral and cellular immune responses (see also p. 1420). Some B lymphocytes mature into plasma cells particularly in the marginal zones, secreting antibodies into the circulating blood when stimulated. T lymphocytes carry out a wide range of defensive activities, described elsewhere in detail (p. 1420). Lymphocytes are also added to the general defence of the body by passing into the haemal circulation via venous sinuses and thus the spleen is an important source of these cells.

When antigenically stimulated, the white pulp increases in size as lymphocytes proliferate; the (primary) follicles become intensely active in B-cell proliferation, and gain the typical appearance of secondary follicles (germinal centres), as in lymph nodes (p. 1432). The presentation of antibody–antigen complexes by dendritic cells of the follicles and marginal zones are involved in these processes and in the generation of immunological 'memory' for future immune responses.

Cytopoiesis

In human fetuses, from the fourth month onwards, the spleen is haemopoietic, the red pulp housing groups of myelocytes, erythroblasts and megakaryocytes. In some anaemias and myeloid leukaemia, stem cells persisting in red pulp may revert to haemopoiesis. In the mature spleen lymphopoiesis in the white pulp contributes to a circulating reserve of immunologically competent T and B lymphocytes and also mononuclear phagocytes.

CLINICAL ASPECTS OF THE SPLEEN

Splenic hypertrophy

In individuals suffering chronic breakdown of erythrocytes, for example in malaria and other haemolytic diseases, the splenic tissues may be permanently hypertrophied and the spleen greatly enlarged (splenomegaly). These changes involve the distension of the reticular spaces of the red pulp with macrophages loaded with damaged red cells or their breakdown products, the proliferation of reticular cells, increases in macrophage numbers and hypertrophy of the fibrous framework. The spleen may increase to several times its normal size, and in children come to occupy much of the abdominal cavity. Similar events occur in various lipoidoses.

Splenectomy. Partial splenectomy is followed by rapid regeneration of lost tissue but even total splenectomy has few obvious effects, its functions being largely assumed by the liver. However, especially in the early years of life, splenectomy may entail a general reduction in the rapidity of immune responses and a consequent increased susceptibility to infection. Splenectomy in later life is followed by leucocytosis with increased lymphocytic, neutrophil, eosinophil and platelet counts in peripheral blood, interpreted as due to removal of humoral factors produced in the spleen which oppose the formation and release of cells from haemopoietic tissues. These effects fade and disappear within a few weeks.

Any massive immune response may be accompanied by splenic enlargement, also occurring in many reticuloses. In splenomegaly, the anterior border, anterior diaphragmatic surface and notched superior border become palpable below the left costal margin; the marginal notches are exaggerated and easily palpable. The transverse colon and left colic flexure are displaced downward, no area of colonic resonance remaining over the enlarged spleen, in contrast to a retroperitoneal tumour (e.g. renal), which does not displace the gut and, therefore, leaves an area of colonic resonance. There is no anastomosis between the smaller splenic arteries so that their obstruction leads to infarction. During splenectomy the tail of the pancreas is in danger.

In addition to the encapsulated peripheral lymphoid organs, lymph nodes and spleen, large amounts of unencapsulated lymphoid tissue exist in the walls of the alimentary, respiratory, reproductive and urinary tracts, and in the skin, termed collectively epithelio-lymphoid tissue. *Skin-associated lymphoid tissue* (*SALT*) also includes the lymphoid tissue of the breast; these are considered elsewhere with the integumental system (p. 424). The other types are usually referred to as *mucosa-associated lymphoid tissue* (*MALT*), subdivided into those situated in the wall of the gut (*gut-associated lymphoid tissue*; *GALT*), the respiratory tract (*bronchial-associated lymphoid tissue*; *BALT*), and the less studied *genitourinary lymphoid tissue* (at present apparently devoid of acronyms). They have a similar structure, although regionally variable and functionally distinct in terms of their specific lymphocyte populations. The most intensively studied are those related to the alimentary tract, and emphasis will therefore be placed on these lymphoid structures, although the general principles apply to other groups (see **9.61**).

MUCOSA-ASSOCIATED LYMPHOID TISSUE

Mucosa-associated lymphoid tissue includes an exceedingly large population of lymphocytes because of the extensive nature of the alimentary tract. The cell aggregations can be divided into two classes: the *organized mucosa-associated lymphoid tissue* (*O-MALT*)

located in the lamina propria and sometimes the submucosa (also known as *lymphoid nodules*), and *diffuse mucosa-associated lymphoid tissue* (*D-MALT*), consisting of numerous cells derived from the O-MALT, scattered throughout the lamina propria and the base of the epithelium. O-MALT includes the peripharyngeal lymphoid ring of tonsils (palatine, lingual, nasopharyngeal and tubal), oesophageal nodules and similar lymphoid tissue scattered throughout the alimentary tract from duodenum to anal canal, although, interestingly, absent from the stomach. There are especially prominent aggregations of nodules in the small intestine (Peyer's patches) and in the vermiform appendix. Bronchial-associated lymphoid tissue (BALT) is the equivalent lymphoid nodular tissue of the lower respiratory tract (of course derived embryonically from the alimentary tract).

ORGANIZED MUCOSA-ASSOCIATED LYMPHOID TISSUE

Although the detailed form of this tissue depends on its location, the basic organization is similar in all regions. Local variations are related to the type of epithelium which they are close to, and to the size of the lymphoid mass. In this account the general principles of their organization will first be described, and then the special features of large aggregations of particular clinical significance, namely the palatine and nasopharyngeal tonsils and Peyer's patches.

General features of O-MALT

Briefly, these are:

- the presence of proliferative centres for B- and T-lymphocyte production (follicles and parafollicular zones, respectively)
- proximity to an epithelial surface, the lymphoid tissue being essentially situated within the mucosal lamina propria
- the lack of a fibrous capsule
- the provision of high-endothelium venules (HEVs) for immigration of lymphocytes
- the presence of efferent lymphatics but virtual absence of afferents.

Follicles and parafollicular zones. B and T lymphocytes are to some extent segregated into distinctive territories within the lymphoid tissue. The B lymphocytes are mainly present in speroidal masses termed *follicles* where they proliferate and undergo maturation. T lymphocytes lie between the follicles in less well-defined *parafollicular zones*, where they also proliferate and begin maturation. The detailed arrangement of cells within these two areas closely resembles that of lymph nodes (see p. 1432 for details). The lymphoblasts (centroblasts) in each primary follicle undergo rapid mitosis to expand the clones of B lymphocytes which then migrate to the periphery of the follicle. The central dividing population and their newly arrived antecedents and newly formed progeny create a pale-staining *germinal centre* (*secondary follicle*) in the middle of the primary follicle. Each germinal centre is surrounded by a ring of closely packed small cells, which migrate predominantly towards the side of the follicle facing the overlying epithelium, to create a densely staining cap, the *mantle zone*. In the central region of the follicle there are follicular dendritic cells—antigen presenting cells (APCs) with long cytoplasmic extensions. Macrophages and a few T cells are also present.

Parafollicular zones. These are more uniform in appearance than the follicles, and consist of loosely packed T lymphocytes of various sizes, some of them mitotic but many small lymphocytes. Amongst these cells are *interdigitating cells*, another form of APC which is characteristic of T-cell areas, and *macrophages*. The parafollicular regions are rich in postcapillary venules with high endothelia, sites of lymphocytic immigration from the bloodstream (see below).

Follicle-associated epithelium (FAE). As noted above, the type of epithelium depends largely on the location of the lymphoid tissue. In the oropharynx and oesophagus it is stratified squamous, in the nasopharynx it is mainly ciliated pseudostratified and in the small and large intestine, simple columnar epithelium. The lymphoid tissue is often invaded by epithelial diverticula in the form of glands or crypts which create a larger area of contact between the two tissues. The FAE covering lymphoid tissue is unusual in possessing cells which are involved in sampling antigens present in the lumen and passing them to the underlying tissues. The main function of B lymphocytes is to produce IgA for secretion into the lumen of the tracts which they line, and so it is essential for lymphoid tissue to 'see' the antigen in order to produce the right antibodies to attack the organisms (and toxins) within the lumen on the other side of the epithelium. The epithelium is able to sample these antigens and translocate them to the antigen-presenting cells of the underlying lymphoid tissue so that appropriate clones of T and B cells can be selected and amplified prior to their migration into the surrounding mucosa. In the small and large intestine these epithelial cells have characteristic short microvilli on their luminal surface and are known as *microfold* (*M*) *cells* (pp. 1769, 1784). In the palatine tonsils they include modified stratified squamous *reticulated epithelial cells* (p. 1446).

Connective tissue framework. Lymphocyte populations are supported mechanically by a fine network of fine collagen (reticulin) fibres and associated fibroblasts, with coarser connective tissue trabeculae in the larger nodules, such as those in the pharyngeal tonsil. There is continuity between the nodules and surrounding tissues as, unlike the lymph nodes, there is no capsule; the lymphocytes can therefore migrate out into the neighbouring regions (although it is thought that they are mainly distributed through vascular channels, as described below).

Vascular routes of cell migration. Another important characteristic of O-MALT is that the lymphatic vessels of nodules are typically only efferent, draining into the lymphatic channels of the organ in which they are sited (although some instances of afferent lymphatics from local areas have also been described: see below).

Lymphocytes migrate into the lymphoid nodules through blood vessels from the surrounding connective tissue. These small arteries and arterioles branch to supply the parafollicular areas with capillary plexuses draining to specialized postcapillary venules whose walls are lined by high endothelium (high endothelium venules; HEVs). These endothelial cells possess adhesion molecules (e.g. vascular cell adhesion molecules; VCAM, see p. 1462) which bind ligands (selectins, p. 1461) on the lymphocyte surface to separate them from the flow of blood and initiate their migration through the vessel wall into the surrounding extravascular spaces of the lymphoid tissue. B and T lymphocytes migrate first into the parafollicular areas where interdigitating APCs also arrive from the bone marrow by the same route. As in the lymph node, T-cell clones are selected by APC–T-cell receptor contact, and the B cells are also activated in the same way. The B cells then migrate to the follicles where they form centroblasts and centrocytes, proliferating and eventually migrating out of the follicle as a primed B cell. Lymphocytes can leave the nodule mainly through the efferent lymphatic drainage and some by direct migration into the surrounding tissues. Some B lymphocytes around the periphery of the nodules mature into plasmacytes to provide IgA and IgG for local defence.

FUNCTIONS OF O-MALT

It can be concluded from the above account that organized mucosa-related lymphoid tissue nodules are secondary lymphoid organs seeded by B and T lymphocytes from primary lymphoid organs (thymus and bone marrow) via the bloodstream. Antigens derived from the neighbouring luminal surface of the neighbouring epithelium (and to some extent from the mucosa itself) are taken up by various APCs, processed and used to select clones of B and T lymphocytes for further proliferative expansion. The majority of lymphocytes resulting from these processes then migrate back into the circulation via the efferent lymphatic drainage, and home to various sites in the tissues related topographically to the nodules. Others may migrate directly into the surrounding tissues, while a few may remain within the nodule itself, to provide local defence. Because mature, antibody-synthesizing B cells (plasmacytes) form part of the latter population, IgA, IgG, IgM and IgE produced by these cells also pass into the efferent lymphatics and contribute to the circulating antibodies of the lymph and blood.

It has also been speculated that lymphoid nodules may contain primary lymphoid tissue responsible for the initial commitment and differentiation of B lymphocytes, in the same way that the thymus does for the T-cell lineage. This idea stems from the observation already noted (p. 1417) that in birds, a diverticulum of the hindgut proctodeum called the bursa of Fabricius houses primary B-cell lymphoid tissue (hence the 'B' of B lymphocytes, originally denoting *bursa equivalent*). This suggested that alimentary lymphoid tissues in

mammals might have a similar function, but experiments have generally failed to support this idea. There is however some recent evidence suggesting that intestinal O-MALT may indeed contain primary B-cell lymphoid tissue during early development, although it is probably of relatively minor importance compared with the bone marrow in the total output of these cells.

DIFFUSE MUCOSA-ASSOCIATED LYMPHOID TISSUE

Diffuse mucosa-associated lymphoid tissue refers to the disseminated population of lymphocytes within the mucosal lamina propria and epithelial base. These have already been selected by APC action in lymphoid nodules and consist of T and B lymphocytes engaged in humoral and cellular immune responses, which have migrated to their final destinations within the circulation. These cells act co-operatively with each other, and also with the local macrophages and other surrounding tissue cells, especially the epithelium to:

(1) maintain the immune barrier functions at the mucosal surface by controlling potential pathogens on the external surface of the epithelium through antibody secretion, and also to eliminate tissue cells which may become infected with viruses or other pathogens (or perhaps have become neoplastic);
(2) engage in more aggressive defence if the epithelial barrier is broken and pathogens invade the lamina propria.

In these actions the lymphocytes are assisted and regulated by macrophages and the overlying epithelia, both of which can act as antigen-presenting, MHC II-positive cells under inflammatory conditions.

B lymphocytes. These are mainly involved in the synthesis of secretory antibodies of the IgA class occurring in alimentary secretions and in IgE (homocytotropic antibody mainly related to mast cell activities, see p. 79). These antibodies are secreted first by plasmacytes in the lamina propria and intercellular spaces of simple epithelia and in the vicinity of subepithelial glands. Antibodies are passed to certain glandular cells (although not goblet cells, at least in the gut) which possess characteristic polymeric IgA receptors on their basolateral surfaces, enabling them to endocytose IgA molecules and pass them into their secretory pathways. During this process the IgA is glycosylated to form *secretory IgA* (*sIgA*) which is then secreted with mucus into the lumen of the viscus they are located in. These antibodies are vital in eliminating pathogenic organisms, although other types of antibody (IgM and IgG), secreted by plasma cells of the lamina propria, are also essential to the destruction of any pathogens which breach the epithelium and infect the adjacent tissues. Some IgM and IgG is also secreted at the surface and can be found in many secretions, for example saliva, milk, etc.

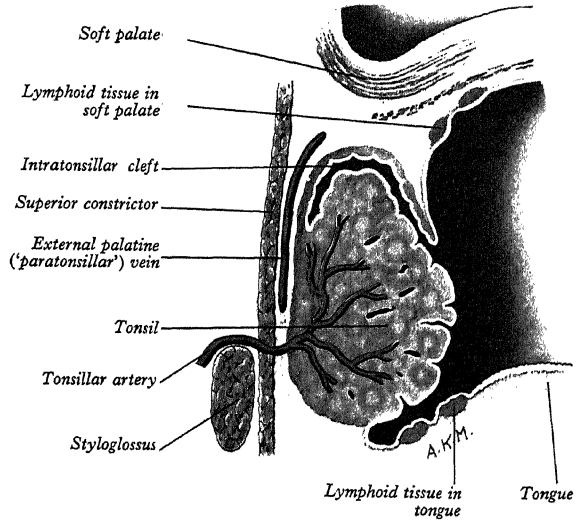
T lymphocytes. These engage in the typical repertoire of this cell type (p. 78), CD4+ helper T cells stimulating B-cell activity, and CD8+ cytotoxic T cells engaging in the destruction of virus- and parasite-infected cells (especially those of the epithelium), and of neoplastic cells, as well as synthesizing various cytokines with complex actions on the lymphocyte population. The surveillance of the epithelium is of obvious importance, because it is a prime target for micro-organisms in the lumen of the gut and at other exposed surfaces, and many of the lymphocytes migrating within the intercellular spaces of the epithelia (see e.g. **12.64**) are T cells.

Lymphocyte homing. The delivery of lymphocytes formed in different types of MALT to their final destinations appears to be fairly specific, and so it seems that some mechanism must exist by which circulating lymphocytes can recognize the tissues or regions of the body they are appropriate for and then migrate into them to play their special part in the immune response. Although it is not certain exactly how specific this homing mechanism is, it appears that lymphocytes from Peyer's patches are seeded into the intestines, those from the BALT into the respiratory system, etc. This seems to be achieved by a system of variable adhesion molecules expressed on the surfaces of lymphocytes (homing receptors) and on the venular endothelium (vascular addressins) within different areas of lymphoid tissue.

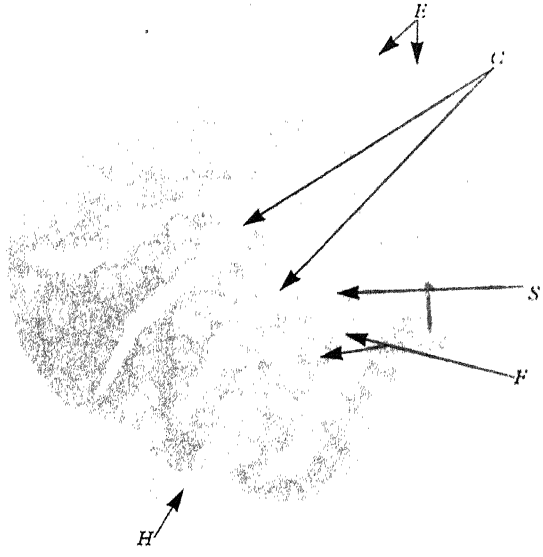
PALATINE TONSIL (9.46–53)

Introduction

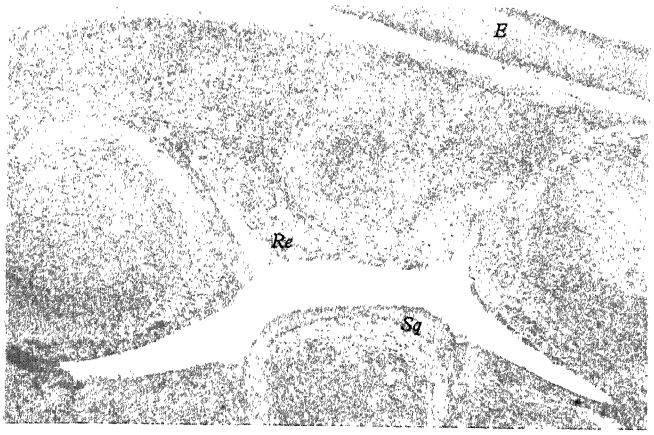
The topographical anatomy of the palatine tonsil is described with the alimentary tract on pages 1728, 1729. In brief, the palatine



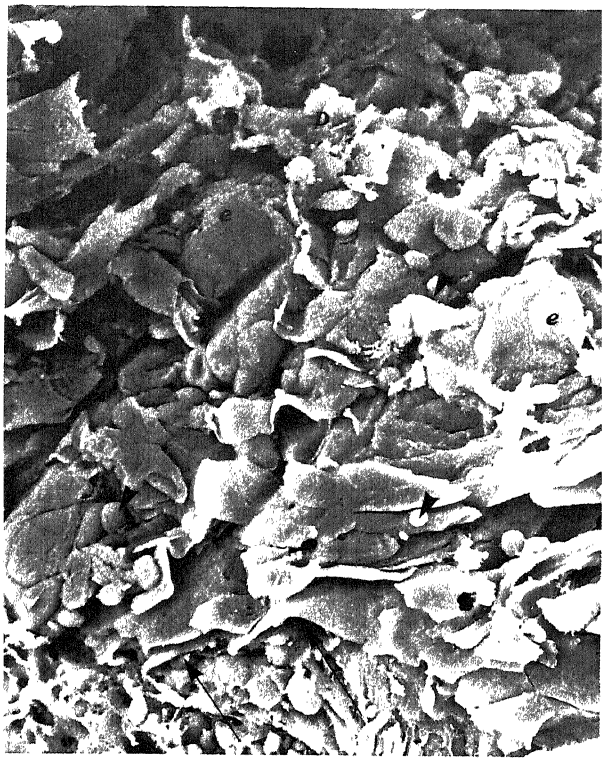
9.46 Coronal section through the palatine tonsil.



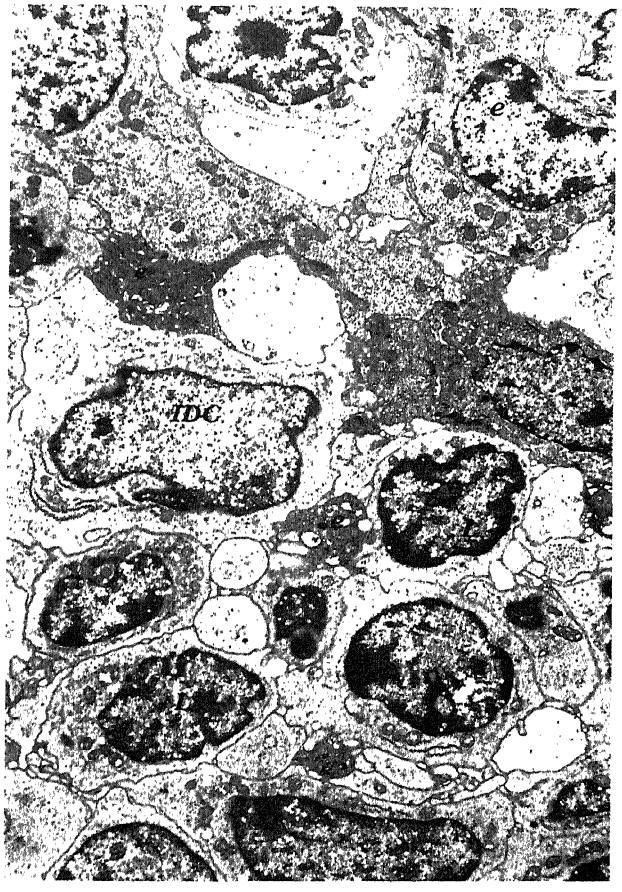
9.47 Transverse section through a whole palatine tonsil, showing many secondary follicles (F) arranged in parallel to the connective tissue septa (S); their dark-staining mantle zones are facing towards the tonsillar crypts (C). Also visible are the oropharyngeal surface epithelium (E) and the connective tissue hemicapsule (H). Haematoxylin and eosin. (Provided by M Perry and photographed by Sarah Smith, Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)



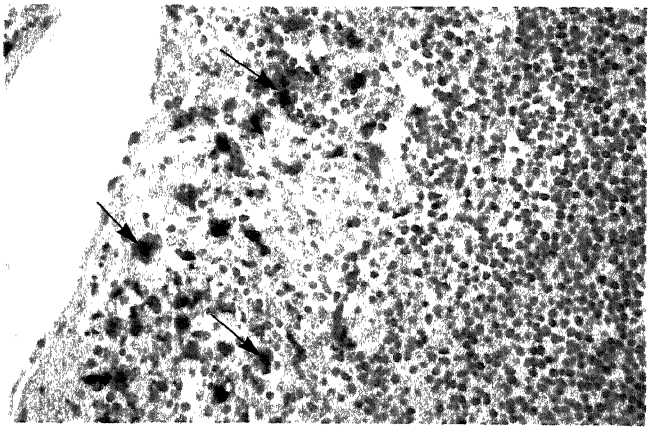
9.48 Palatine tonsil: transected tonsillar crypt lined with patches of stratified squamous (Sq) and reticulated (Re) epithelium, contrasting with the thick epithelial covering of the oropharyngeal surface (E). Haematoxylin and eosin. (Provided by M Perry and photographed by Sarah Smith, Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)



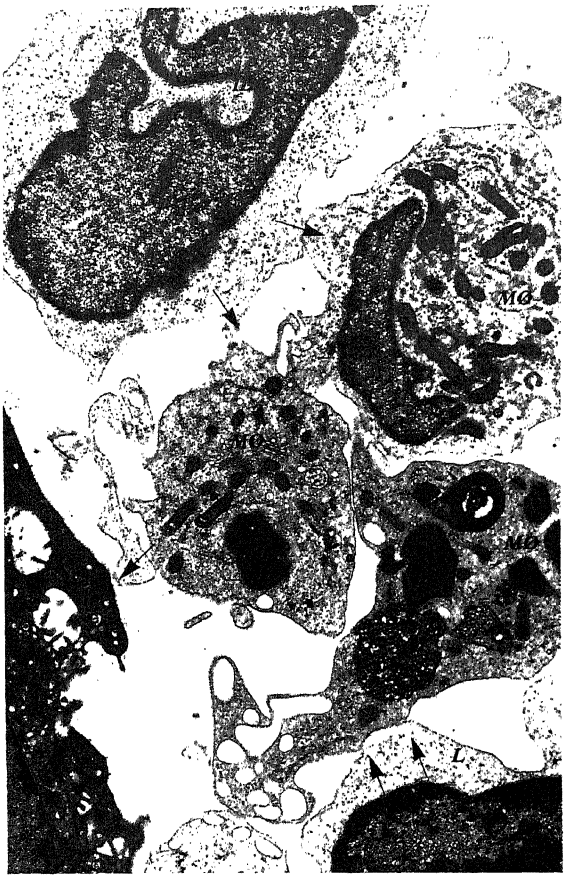
9.49 Scanning electron micrograph of the reticulated crypt epithelium (e) in a palatine tonsil, showing the tonsillar surface broken open to reveal the lymphocytes and other non-epithelial cells (arrowheads) within the cavities at the base of the attenuated stratified squamous epithelium. Also shown is cellular debris (D) within a crypt. (Provided by M Perry, Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)



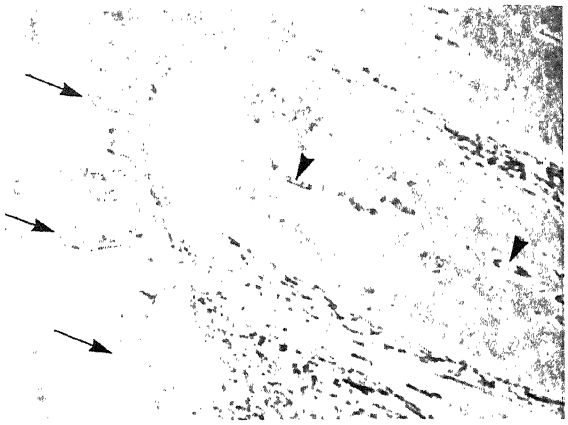
9.50 Transmission electron micrograph of a palatine tonsil through part of an interfollicular area showing interdigitating cells (IDC) and lymphocytes (L). The processes of a few epithelial cells (e) are also visible. (Provided by M Perry, Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)



9.51 Reticulated epithelium from a crypt of a palatine tonsil, immunostained to show numerous interdigitating cells and macrophages, identified by their immunostaining for the S-100 antigen. Note the close contacts between these cells and infiltrating lymphocytes (arrows). Immunoperoxidase stain on a paraffin section. (Provided by M Perry, Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)



9.52 Transmission electron micrograph of an area of reticulated epithelium of a palatine tonsil, showing intimate contacts (arrows) between macrophages (Mϕ), an interdigitating cell (asterisk) and a lymphocyte (L). (Provided by M Perry, Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)



9.53 Palatine tonsil in section, stained with silver to demonstrate the fine reticulin network supporting the lymphoid tissue. Reticulin is seen to outline the undulating basement membrane of the non-keratinized stratified squamous epithelium covering the oropharyngeal surface (arrows). Reticulin also forms a lattice in the interfollicular areas, and also supports the tonsillar microvasculature including the follicular vessels (arrowheads). Gordon and Sweet silver method. (Provided by M Perry, Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)

tonsils are bilateral almond-shaped masses (hence the Greek term *amygdala* = almond) situated in the oropharynx within the tonsilar recesses, between the palatoglossal and palatopharyngeal folds. Each is a mass of lymphoid tissue covered on its oropharyngeal aspect by non-keratinized stratified squamous epithelium. It is supported internally by connective tissue septa and a network of finer fibres, continuous with the hemicapsule of the tonsil which forms its lateral boundary with the oropharyngeal wall, and with the mucosa covering its free surface (**9.46**). 10–30 or more *crypts* are formed by the invagination of the latter surface. These are narrow tubular epithelial diverticula which often branch within the substance of the tonsil. The epithelium lining the crypts is in part similar to that of the general surface, i.e. stratified squamous in type, but there are also patches of *reticulated epithelium*, a much thinner tissue with a complex structure, and of great importance in the immunological function of the tonsil.

Reticulated epithelium

The reticulated epithelium (**9.47, 48, 50–52**) lacks the orderly laminar structure of the stratified squamous epithelium, its base being deeply invaginated in a complex manner, so that the epithelial cells, with their slender branched cytoplasmic processes, provide a mesh with large interspaces to accommodate the infiltrating lymphocytes and macrophages. The basal lamina of this epithelium is discontinuous. Although the oropharyngeal surface is unbroken, the epithelium may become exceedingly thin in places, with only a tenuous cytoplasmic lamina separating the pharyngeal lumen from the underlying lymphocytes. Epithelial cells are held together by small desmosomes, anchored into bundles of keratin filaments. Langerhans' cells and interdigitating APCs are also present within the reticulated epithelium. The intimate association of epithelial cells and lymphocytes, often referred to as 'lympho-epithelial symbiosis' (Fioretti 1957), is particularly well designed for the direct transport of antigen from the external environment to the tonsillar lymphoid cells (Brandtzaeg 1988; Perry et al 1988) so that the reticulated epithelial cells are thought to be functionally similar to the microfold cells of the gut (p. 1769). The total surface area of the reticulated epithelium is very large because of the complex branched nature of the tonsillar crypts, estimated at 295 cm^2 for an average palatine tonsil (Slipka & Kotyza 1987). It is noteworthy that, in contrast, lymph nodes depend on indirect antigenic delivery through *afferent lymphatic vessels*, which are absent from the tonsil (although efferent lymphatics drain it, like other examples of MALT).

It is noteworthy that the crypts also often contain desquamated epithelial cells mixed with intact and degenerating lymphocytes, occasional erythrocytes, cellular debris and, sometimes, micro-organisms. The degeneration of epithelial cells is frequently accompanied by the formation of small spheroidal clusters of stratified squamous epithelium known as *tonsillar corpuscles*, especially in the narrower rami of the crypt system (Slipka & Kotyza 1987; Perry & Slipka 1993). These closely resemble thymic epithelial corpuscles (of Hassall, see p. 1426) and may represent sites of accidental separation of the pharyngeal epithelium from the tonsillar surface rather than any functional significant features of the tonsil.

Lymphoid tissue (9.49–52)

The tonsillar lymphoid tissue can be divided into four lymphoid compartments participating in immune responses (Brandtzaeg 1988). These are:

(1) lymphoid follicles with germinal centres, rounded cellular aggregations consisting mainly of B-lymphocytes and their precursors, scattered follicular dendritic cells and some macrophages with radiating extensions ('starry sky macrophages'). Germinal centres are arranged in rows roughly parallel to neighbouring connective tissue septa. Their size and cellular content varies in proportion to the immunological activity of the tonsil;
(2) the mantle zones of the lymphoid follicles, each with closely packed small lymphocytes forming a dense cap, always situated on the side of the follicle nearest to the mucosal surface. These cells are the products of B-lymphocyte proliferation within the germinal centres;
(3) extrafollicular, or T-lymphocyte areas containing specialized segments of microvasculature including high endothelial venules (HEVs), through which circulating lymphocytes enter the tonsillar parenchyma (Perry et al 1992a,b).

(4) the lymphoid tissue of the reticulated crypt epithelium containing predominantly IgG- and IgA-producing B lymphocytes (including some mature plasmacytes), T lymphocytes and antigen-presenting Langerhans cells. In this subsurface region there are also numerous capillary loops, and in some heavily reticulated areas, HEVs with transmigrating lymphocytes (Perry et al 1988, 1992b).

Connective tissue framework

The whole of the tonsil is supported by a delicate meshwork of fine collagen (reticulin) fibres secreted by their associated fibroblasts (9.53). The collagen fibres are condensed in places to form more robust connective tissue septa also containing elastin, creating a series of partitions within the tonsillar mass, the follicles being placed on either side of septa (9.46). The septa merge at their ends with the dense irregular fibrous hemicapsule on the deep aspect of the tonsil and with the lamina propria on the pharyngeal surface. Blood vessels, lymphatics and nerves branch or join within the connective tissue condensations.

Vascular systems

Blood vessels. These have been studied in detail in microcorrosion casts of the human tonsil by Ohtani et al (1989). Arteries enter the deep surface branch within the connective tissue septa, narrow to become arterioles and then give off capillary loops into the follicles, interfollicular areas and into the cavities within the base of the reticulated epithelium (see above). The capillaries rejoin to form venules, many with high endothelium, as already noted, and the veins return within the septal tissues to the hemicapsule as tributaries of the pharyngeal drainage. For further details of the macroscopic aspects of the vasculature, see page 1729.

Lymphatics. Efferent lymphatics arise in dense plexuses of fine capillaries surrounding each follicle. These join to form larger lymphatics which eventually exit through the connective tissue hemicapsule and thence through the adjacent superior constrictor to the nodes of the deep cervical lymphatic drainage (p. 1729).

Tonsillar functions

Like other mucosa-associated lymphoid masses, the major functions of the palatine tonsils are as follows (see Brandtzaeg 1988):

- to select clones of B and T cells relevant to the micro-organisms at the pharyngeal surface. To initiate this action it is envisaged that antigens cross the reticulated epithelium and are passed on to antigen-presenting cells which carry out T- and thus B-cell selection;
- to provide a site for the proliferative expansion of selected B- and T-lymphocyte clones destined for immune functions in neighbouring areas of the pharyngeal mucosa;
- to produce IgA and IgG for local secretion (apparently a minor function which may be primarily concerned with the immediate protection of the tonsil itself).

Palatine tonsils belong to the class of secondary lymphoid tissue, and the precursors of the proliferating B- and T-lymphocyte populations originate in the primary lymphoid tissues, i.e. the bone marrow and thymus, respectively. Entering the tonsils by migration across the walls of the HEVs, the two classes of lymphocytes move to their specific areas, and proliferate when suitably stimulated, under the influence of APCs and macrophages. After T-cell contact with an APC cell and B-cell stimulation, lymphocytes leave the tonsil in the efferent lymphatics, and probably also to some extent by direct peripheral migration into the mucosa of the surrounding pharynx. It is thought that those which leave by the lymphatic route and thus pass into the general circulation eventually migrate to the pharynx and adjacent areas of mucosa through venules and become important in local defence, including the secretion of IgA through mucoserous glands, immune surveillance and other related activities.

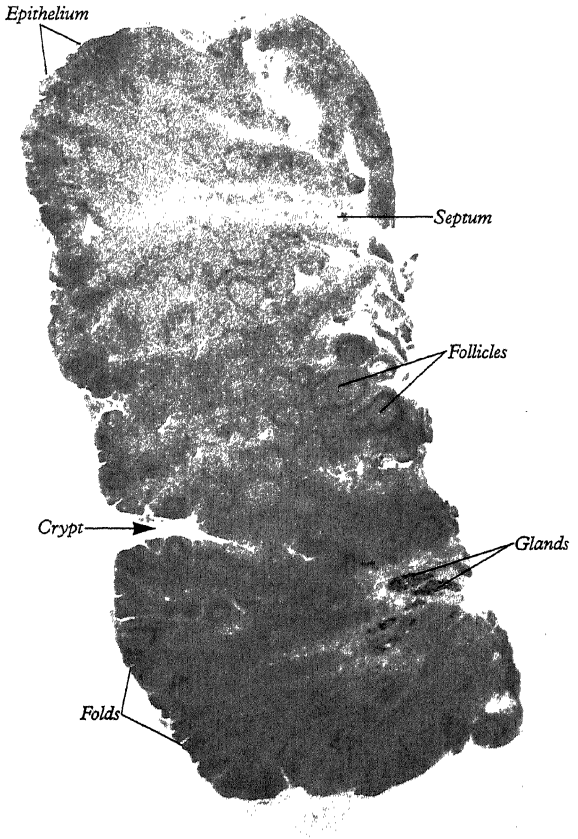
Tonsillar pathology

While the palatine tonsil is a substantial part of the pharyngeal immune system, it may itself become infected; in particular, pathogenic bacteria, for example streptococci, may invade the tonsillar crypts and proliferate within them, causing an inflammatory reaction including the migration of leucocytes into the cryptal spaces. Various factors including the expansion of germinal centres cause swelling of the tonsillar mass, and the pus within the crypts is visible as yellowish spots on its inflamed surface. Tonsillectomy after repeated episodes of tonsillitis might be expected to cause considerable reduction of pharyngeal defence, but this usually does not appear to be the case, probably because other related lymphoid tissue masses, for example the lingual tonsil, increase their lymphocytic output.

NASOPHARYNGEAL TONSIL (ADENOIDS)

The nasopharyngeal tonsil is a median tonsillar mass, situated in the roof of the nasopharynx; it has many resemblances to the palatine tonsil in its cellular organization and functions, although there are also some differences, mainly because it is situated in the nasopharyngeal mucosa rather than that of the oropharynx. Its macroscopic anatomy is described with the alimentary tract (p. 1728), and the present account will be mainly restricted to its microstructure.

The nasopharyngeal tonsil at its maximal size (during the early years of life) is shaped like a truncated pyramid hanging from the nasopharyngeal roof. It consists of a mass of lymphoid tissue embedded in the mucosa of the nasopharynx (9.54), and is thus covered at its sides and below mainly by ciliated respiratory epithelium, although small patches of non-keratinized stratified squamous epithelium also occur. The superior surface is separated from the periosteum of the sphenoid and occipital bones by a connective tissue hemicapsule to which the fibrous framework of the tonsil is anchored. This forms what is described by Oláh (1978) as a three-dimensional labyrinth formed by supportive reticular fibres connected to both the basal lamina and the connective tissue hemicapsule, and filled with lymphoid parenchyma. The epithelium lines

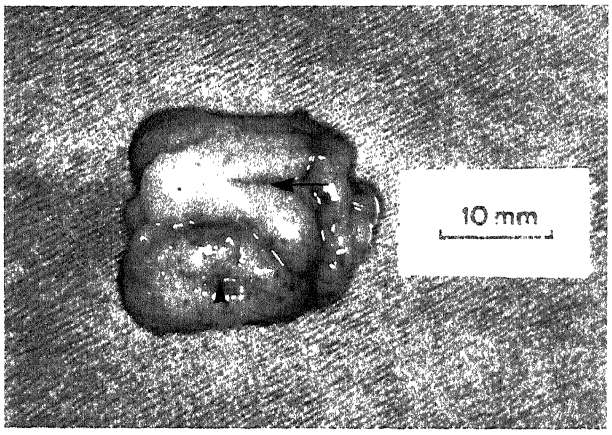


9.54 Transverse section of a nasopharyngeal tonsil. Note numerous lymphoid follicles (F); epithelium with folds (arrows) and the deep crypts (C). L = lacuna; S = scromucous gland; CT = connective tissue septa. Haematoxylin and eosin. Magnification ×9. Provided by N Kirkpatrick and photographed by Sarah Smith (Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London).

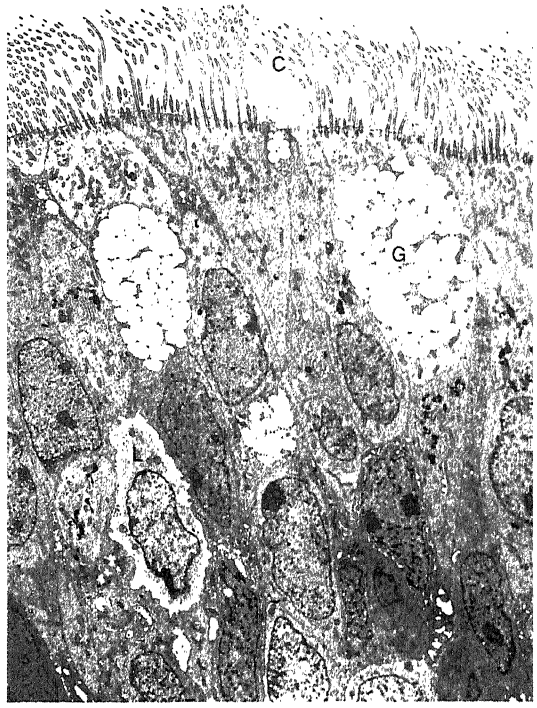
a series of mucosal folds and crypts around which the lymphoid parenchyma is organized into follicles and extrafollicular areas.

The nasopharyngeal tonsil is subdivided into four to six lobes by connective tissue septa, which arise from the hemicapsule and penetrate into the lymphoid parenchyma (**9.54**). Located within this connective tissue are some seromucous glands with their ducts extending through the lymphoid tissue to the cryptal or nasopharyngeal surface (Barnes 1923; Eggston & Wolf 1947).

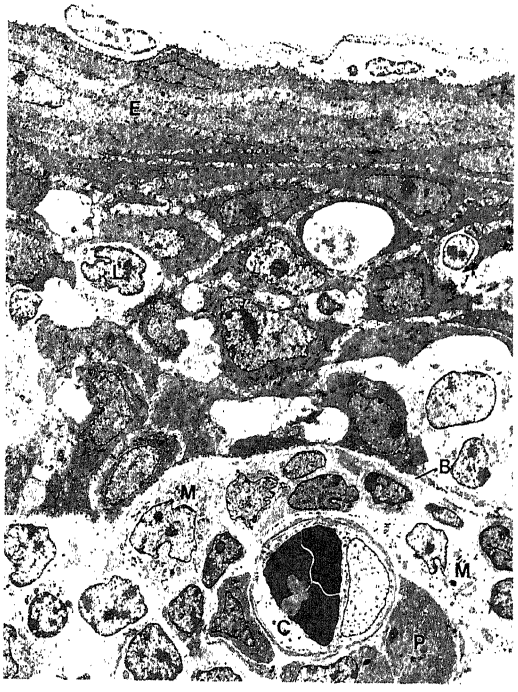
Tonsillar crypts (**9.55–58**). There has been a long standing debate on whether the nasopharyngeal tonsil really possesses true crypts



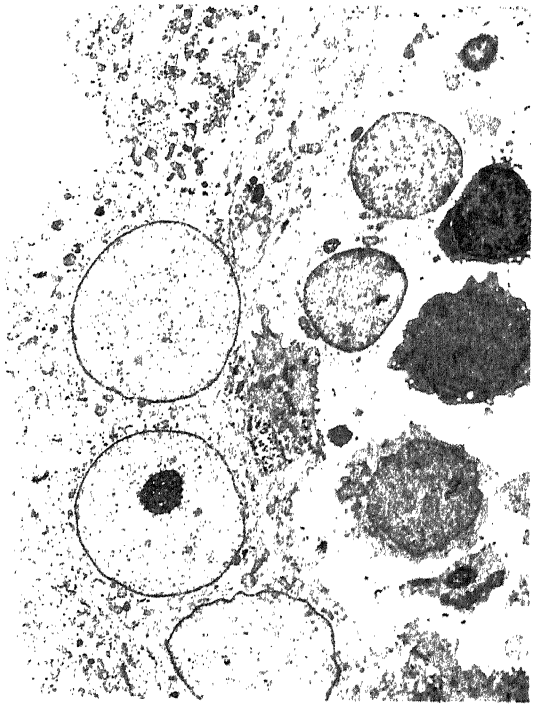
9.55 Appearance of a nasopharyngeal tonsil following adenoidectomy by curctage. Rostral surface is to the left; surface folds radiate forward from a median recess (arrowhead). In this example, the impression left by contact with the left Eustachian cushion is evident laterally (arrow). Specimen provided by M J Gleeson (ENT Department, Guy's Hospital, London).



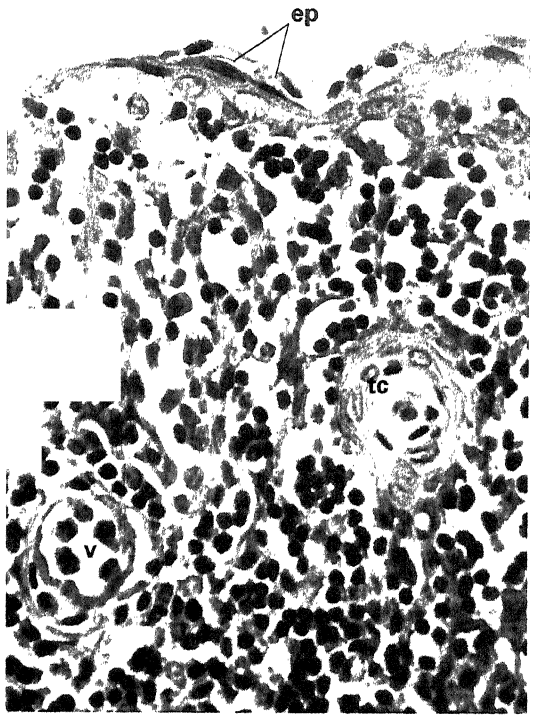
9.56 Transmission EM of a section through the surface of a nasopharyngeal tonsil, showing pseudostratified epithelial cells with cilia (C), a goblet cell (G), and an intraepithelial lymphocyte (L). Magnification × 4000. Specimen provided by N Kirkpatrick and photographed by Sarah Smith (Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London).



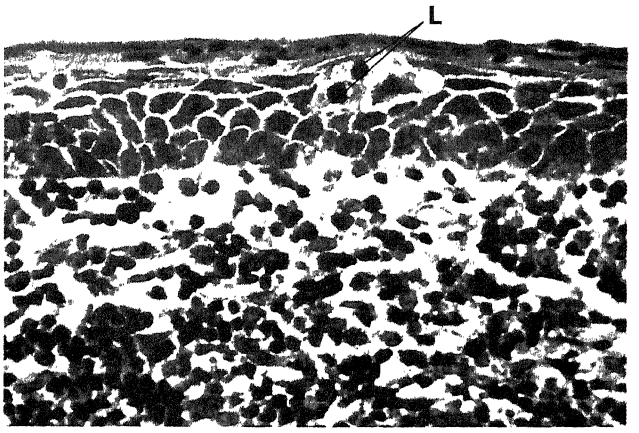
9.57 Transmission EM of stratified squamous epithelium at the nasopharyngeal surface, with macrophages (M), a capillary (C), plasmacyte (P) in the lamina propria and basal lamina (B). Magnification × 2500. Specimen provided by N Kirkpatrick, and photographed by Sarah Smith (Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London).



9.58 Transmission electron micrograph of the nasopharyngeal surface showing intermediate epithelium, with lymphocytes at its base. Magnification × 6500. Specimen provided by N Kirkpatrick, and photographed by Sarah Smith (Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London).



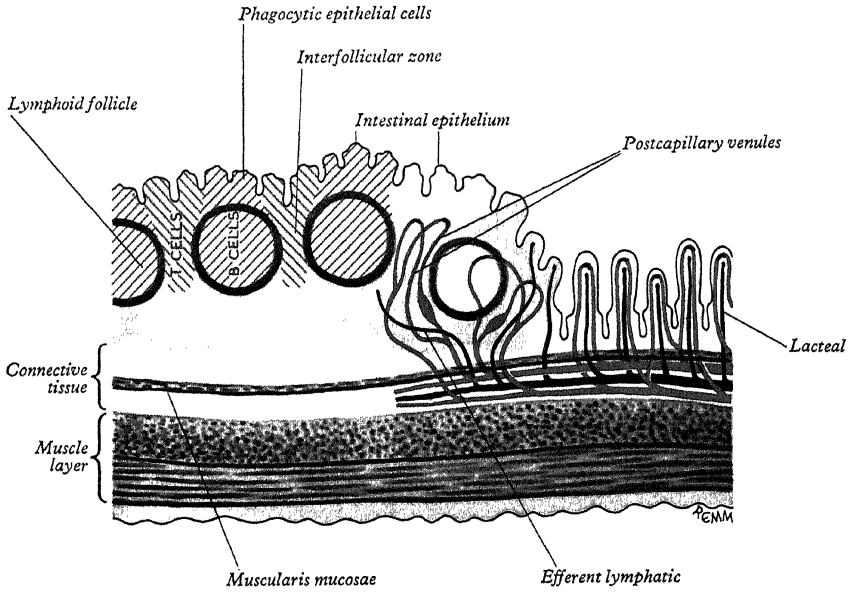
9.59 Reticulated stratified squamous epithelium of a nasopharyngeal tonsil, with network of intraepithelial channels containing many non-epithelial cells. Note also: tonsillar corpuscle (tc) and an intraepithelial venule (v). PAS stain. Magnification × 640. Provided by N Kirkpatrick, and photographed by Sarah Smith (Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London).



9.60 Section through surface of the nasopharyngeal epithelium showing a patch of stratified squamous epithelium with intraepithelial lymphocytes (L), subepithelial lymphocytes and capillaries. Movat's stain. Magnification × 250. Provided by N Kirkpatrick and photographed by Sarah Smith (Division of Anatomy and Cell Biology, UMDS, Guy's Campus, London).

like those of the palatine tonsil, or merely a deeply folded surface. Ali (1965a,b) and Kirkpatrick et al (1993) describe the presence of both numerous superficial folds and deep, sometimes branching crypts extending right through the nasopharyngeal tonsil almost to the connective tissue hemicapsule. The epithelium lining the folds and crypts, and that covering the nasopharyngeal surface is not uniform and there are patches of pseudostratified ciliated, non-keratinized stratified squamous, and occasionally a simple cuboidal, epithelium in all locations. Pseudokeratinization occurs rarely in deep branching crypts. However, in all three of these types of epithelial cover there are patches of typical *reticulated epithelium* of variable size and depth, as in the palatine tonsil (see above, and **9**.58–59). The degree of reticulation varies so that in some sites only a few lymphocytes and other non-epithelial cells infiltrate into the epithelial base, and in others there are dense cellular aggregates, resulting in the loss of the ordered epithelial architecture. This reticulated epithelium also frequently becomes vascularized (Kirkpatrick et al 1993).

The internal structure of the nasopharyngeal tonsil closely resembles that of the palatine tonsil, and the same lymphoid compartments can be discerned, i.e. follicles with germinal centres and mantle zones containing B lymphocytes, follicular dendritic cells and macrophages; extrafollicular areas with T lymphocytes and interdigitating cells; and the reticulated epithelium. HEVs also exist within the extrafollicular tissue, indicating similar routes of lymphocyte entry and dispersal (**9**.60).



9.61 The organization of an epitheliolymphoid complex in the wall of the small intestine (a Peyer's patch), showing the distribution of B lymphocytes associated with the follicles and T lymphocytes in the interfollicular zones; the arrangement of the vascular supply of the lymphoid tissue is also shown (right).

Functions of the nasopharyngeal tonsil

The general contribution of the nasopharyngeal tonsil to the defence of the upper respiratory tract is probably similar to that of the palatine tonsils and other parts of the circumpharyngeal lymphoid ring. The territories served by its lymphocytes are uncertain, but may include the nasal cavities, nasopharynx, pharyngotympanic tubes and the middle and inner ear which are topographically related areas. For clinical aspects, see page 1729.

PEYER'S PATCHES

Introduction

Peyer's patches are aggregations of O-MALT which form domelike elevations of the mucosa present throughout the small and large intestines. In the ileum they often form quite large areas of lymphoid tissue, up to 1 cm wide by 2 cm long (or sometimes larger), orientated with their long axes parallel to that of the intestine (**12**.111). The name Peyer's patch was originally given to the enlarged masses of lymphoid tissue found in the ileum during typhoid infections, but it is now usually applied to any group of lymphoid nodules in the wall of the small or large intestine including the appendix. The topography of a Peyer's patch is outlined on page 1771.

Microstructure of Peyer's patches (9.61)

In general plan Peyer's patches resemble other masses of O-MALT (see above) such as the tonsils.

Lymphoid tissue. This is contained mainly within the lamina propria, although large lymphoid masses can extend into submucosal tissues, but its presence causes the overlying epithelium to form a low convex elevation over the region. Within the lymphoid tissue are variable numbers of lymphoid follicles and parafollicular areas similar to those of the tonsil, including the presence of an epithelium-directed accumulation of small lymphocytes (mantle zone) on the follicle periphery, and, in active tissue, germinal centres. HEVs are also features of the parafollicular areas.

Follicle-associated epithelium. The domed surface is generally devoid of villi and crypts, but is covered by a unique epithelium of low columnar cells with short irregular microvilli, interspersed with characteristic *microfold (M) cells*. These are rather flat cells the bases of which are invaginated to form pockets which lymphocytes and APCs can enter in much the same way as in the reticulated epithelium of the tonsils. On their apical surfaces, M cells have many short ridges and stubby microvilli, and between these are deep endocytic pits and vesicles. The details of M cells and other epithelial covering cells vary with position (see Clark et al 1994). In the appendix the lymphoid tissue consists of a continuous layer around the narrow lumen, penetrated by numerous crypts (tubular intestinal glands).

M cells are highly endocytic, and can rapidly transfer material taken into vesicles at their luminal surface through the underlying tissues (transcytosis), releasing them into the intraepithelial pockets at their bases where APCs and T cells are present. In this way, antigenic material in the lumen of the gut can be sampled and presented via APCs to lymphocytes more deeply placed in the mucosa, where appropriate effector action can be taken. The M cells are particularly adhesive to carbohydrates of bacterial cell walls, and may indeed transport whole bacteria and viruses to the underlying tissues. Although this ability is no doubt a great advantage in eliciting powerful immune reactions against them, some pathogens unfortunately overpower the defensive mechanisms within the lymphoid tissue to spread within the body's tissues, for instance, poliovirus, which can gain access to the nervous system through enteric nerves, and *Salmonella typhi*, a bacterial pathogen of the gut wall tissues.

Immune functions

The result of normal antigen sampling and presentation is the selection and proliferation of suitable IgA-secreting B-cell clones which then disseminate through the efferent lymphatics and the systemic blood circulation to the D-MALT population in a much wider area of the alimentary mucosa. There the B cells secrete antibody for transport to the lumen. The antibody they secrete is IgA in the form of dimers which after release from the plasma cells can bind to polymeric IgA receptors on the basolateral surfaces of gland cells and enterocytes. These cells then endocytose the IgA dimers, and after attachment of the secretory component in the Golgi apparatuses of these cells, they are secreted into the lumen as secretory antibody, sIgA. This configuration of dimeric sIgA is highly resistant to enzymatic degradation, and is, therefore, very suitable for action within the hostile, protease-rich environment of the intestinal interior.

There are also various other routes for epithelium–lymphocyte interactions in the gut wall. The enterocytes themselves are MHC-II positive and can act as APCs to T lymphocytes, although these initiate suppressor activities, suggesting that the main importance of this pathway is to induce tolerance of epithelial antigens. Another possible entry point for antigens is the lymphatics. As elsewhere in O-MALT, these are primarily efferent and serve to take lymphocytes away from the lymphoid tissue. However, recent studies in various mammalian species indicate that afferent lymphatics arising in the villi and neighbouring parts of the lamina propria overlying the lymphoid tissue form a plexus of perifollicular sinuses and might, therefore, be involved in the passage of antigen from neighbouring tissues into the lymphoid structure (see Lowden & Heath 1992), although the functional significance of this arrangement is not known.

Because the surface area of the gut is greater than in all other areas of the body the total number of lymphocytes within its walls is substantial.

For further details of Peyer's patches, see the stimulating review by Kraehenbuhl and Neutra (1992).